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PROCEEDINGS

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Hunger and the Hypothalamus

By Jean Mayer*

THE CONTROL of appetite has become a compulsive preoccupation in America today. Open any newspaper and you will find in the women's section, the food section, the medical section—not to mention the ads—any amount of advice on how to lose weight and how to keep reduced without experiencing any unpleasant feeling and without having to exercise in the process. The radio, television and other "mass information" media are equally generous in the sharing of their information. Needless to say, much of this campaign is ineffectual and indeed often harmful, since it is based on misstatements. Some of these statements serve an obvious commercial purpose, such as the selling of "health foods," "low calorie dishes," "fabulous formulas," and the like, while some of them even take the reader deep into a magical region where such natural laws as that of the conservation of energy are abolished, thanks to the persuasive power of a particularly engaging popular lecturer: "Eat all you want and stay thin by following So-and-So's regimen."

Clinicians, of course, recognize these false claims for what they are. But too often there is a tendency among them to underestimate all aspects of the problem of appetite except self control and to assume that food intake in man is regulated purely by will power. Many seem not to experience any particular humility before unsolved physiologic and psychologic difficulties in this field. This attitude calls to mind the famous quip, "We know all the answers, it is the questions we do not know," and may justify summarizing some of the observations pertinent to the problem of food intake which have been observed in the past few years.

During the course of the nineteenth century a number of scientists had tackled the problem—in writing only. Some, such as Erasmus Darwin, Johannes Müller, and Weber, following Haller, saw in hunger a "central" phenomenon: a part of the brain, sensitive to the depletion of its own reserves, gave a warning signal—hunger—to the rest of the higher centers. Others, such as Magendie, Tidewald, and Milne-Edwards, believed that hunger had a "peripheral" origin: an outlying organ—say, the stomach—was the exclusive seat of hunger phenomenon and feelings. Others, like Roux and Michael Foster, considered hunger to be a "generalized" sensation with all organs, the circulating blood and the brain participating in its inception. Just before World War I, the "peripheral" theory appeared to receive experimental confirmation. Walter Cannon at Harvard demonstrated that if a subject was made to swallow a rubber balloon linked to a pressure recording device, prolonged deprivation of food and hunger feelings were accompanied by rhythmic contractions of the stomach. Indeed, the sensation of "hunger pangs" coincided with the occurrence of a number of consecutive and particularly energetic contractions of the stomach walls.

Anton Carlson in Chicago soon confirmed this finding and, in a long series of experiments, related in his book "The Control of Hunger in Health and Disease," he made these gastric contractions the basis of an entire theory of the regulation of appetite. According to him, not just hunger pangs but the whole hunger-satiety complex was determined by the presence or absence of activity in the gastric musculature. He further hypothesized that this activity was largely determined by the level of circulating blood sugar: administration of insulin, which lowers the blood glucose level, soon brings about rhythmic contractions of the stomach and, until relieved by food or by glucose administration, intense feelings of hunger.

This theory not only evoked expert interest, but it achieved almost immediate popular acclaim; here was something easy to understand, which clearly corresponded to the subjective experience of at least some individuals. To be sure, a large number of persons never experience sharply localized hunger pangs; they simply feel "empty" or "hungry all over," or even get headaches when food is not forthcoming on time. But this did not appear to Carlson to represent an over-riding objection.

However, experimental difficulties of a much more insurmountable nature began to accumulate. For example, Morton Grossman and his associates, also working in Chicago, showed that denervation of the stomach in human subjects did not have the consequences as regards appetite that Carlson's theory would have made one anticipate. Vagotomies,

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No references are given, but the reader will find pertinent information in the bibliography listed at the conclusion of the present article.

which eliminate gastric "hunger contractions," and splanchnectomies, which eliminate consciousness of hunger contractions, did not eliminate or even diminish hunger sensations or even delay appearance of hunger as the time of the last meal receded. Those of the patients (a minority) who before the operation had felt definitely localized hunger pangs in the pit of the stomach had simply joined the majority in feeling empty or "hungry all over"; details of their mode of awareness of hunger had been modified, but the central fact remained unchanged.

Another piece of evidence against the central role of the stomach in the "control of hunger" was obtained by E. F. Adolph and his co-workers at the University of Rochester. These physiologists were interested in possible influence of "bulk" as such on appetite and food intake. If an empty stomach and its contractions were the determining factors in the control of hunger, then diluting the diet with inert, indigestible material such as a fine clay or cellulose shavings should decrease the amount of food consumed by animals during the course of a day. Indeed, a number of preparations sold in large (and profitable) amounts at the present time promise easier slimming and decreased appetite on precisely this "filling up" basis. However, in Adolph's experiments, diluting the food with inert material had no more than a transient effect. The animals quickly adjusted for the decreased caloric content per unit volume by consuming more of the diet and once again, stomach sensations were shown to play only a minor role. As for the possible role of blood sugar levels as such in determining appetite, this too was discarded when several workers, Scott and his co-workers in particular, found themselves unable to relate variations in absolute blood glucose levels to hunger sensations. The hunger due to acute hypoglycemia following administration of insulin was considered by many workers to be an emergency phenomenon, unrelated to the normal mechanism. While the existence of "hunger" stomach contractions has thus been demonstrated, and while they play some role in the awareness of hunger in some individuals, they appear to be at best only a partial and secondary aspect of the problem of appetite.

Meanwhile, attention had become focused on the hypothalamus. The reader is of course familiar with Frohlich's misconception of the role of the pituitary in causing what was later shown by Erdheim and Camus and Roussy to be hypothalamic obesity.

The development by Horsley and Clarke of a relatively inexpensive instrument which permits the reproducible placement of small electrocoagulation lesions was an essential factor in the determination of the precise areas responsible for the control of food intake. Hypothalamic obesity in the rat was studied by a number of laboratories, in particular by Dr. John Brobeck at Yale. Using a similar instrument at Harvard, we induced hypothalamic obesity in the mouse, after lengthy miniature work to identify the proper area. Hypothalamic obesity has also been studied in the cat, the dog and the monkey. The use of the stereotaxic instrument has made it possible to show that when symmetrical lesions centered on the ventromedial hypothalamic areas are produced, the injured animals become grossly hyperphagic and accumulate considerable amounts of fat ("active" or "dynamic" phase of obesity) until their weights finally stabilize at a very high level ("passive" or "static" phase of obesity). The weight of the obese animals can be reduced by the simple trick of cutting down on their food intake. But if the "reduced" animals are granted anew free access to food, their weight not only goes up again but does so at a faster rate than during the initial dynamic phase.

The use of the stereotaxic instrument permitted another important advance. An Indian associate of Dr. Brobeck, Bal K. Anand, was able to show that the bilateral destruction of two more lateral hypothalamic centers caused rats to stop eating altogether. Delgado, working at Yale on monkeys, and Larsson, working at Stockholm on goats, also showed that electric stimulation of the same lateral hypothalamic areas induce feeding behavior in the stimulated animals. Such a finding obviously gave an anatomic basis for a possible central mechanism "regulating" food intake, that is, adjusting intake to the requirements of the organism.

For there is such an adjustment. After all, most adult animals and persons maintain their weights at the same level, day in and day out, for years sometimes, in spite of great variations in energy expenditures. Appetite must therefore be adapted to caloric needs in adults. Similarly, growing animals and children grow at well defined rates in spite of the variability of calories expended in exercise, in maintaining the body at the same temperature in spite of changing weather, etc. Again, there must be an adjustment of intake to requirements during the growth period. Cowgill of Yale had been the first to demonstrate that under a variety of circumstances and on a variety of diets, "animals eat for Calories." André Mayer and his co-workers in Paris pursued the problem further: they systematically changed the energy expended by experimental animals by changing the environmental temperature and demonstrated that animals varied their food intake in a way which indicated that two regulations were at work; the first and the most important one adjusts from day to day the calories eaten to the calories spent; and another regulation, much slower, seems to correct over a period of time whatever error the rapid mechanism could have been guilty of. The French workers further showed that the regulation is much more precise at high expenditure levels than at low ones.

The problem of the mechanism of this adjustment remains: if among the many factors that affect food intake there is one relating to metabolic requirements—a factor with a "memory," which can therefore be "regulatory"—on what set of centers does it act, and by what mechanism?

A clear answer to the first question was yielded by the application to the problem of food intake of a well-known behavioral method, developed by Professor Skinner of Harvard for other purposes, in particular the study of sex drive. This adaptation was achieved by my associate, Dr. James Anliker. The rats or mice are put in a cage with a lever; pushing the lever frequently enough is rewarded according to a pre-arranged "schedule" by delivery of a small food pellet. Complete electrical recording gives a faithful picture of the feeding behavior of the animal under observation. This behavior can be followed, mouthful by mouthful, for days and even weeks on end if necessary. Application of this method showed that the feeding behavior of normal rodents (like that of men) exhibits a clear-cut daily pattern. In the case of mice, for example, there is a period of several hours (in the afternoon and evening) during which the feeding is rapid and frequent; this is followed by a "satiety period," during which the animals eat very much less and in a desultory fashion. By contrast, animals in which the ventral area of the hypothalamus has been destroyed with the aid of the stereotaxic instrument or by goldthioglucose (see below) no longer show such a satiety period. The rate at which the mice obtain and consume the small food pellets (this speed can be considered a behavioral measurement of hunger) is not increased over and above the maximum normal rate characteristic of the period of intense eating in normal animals; but the gradual "satiety" tapering is eliminated. This and other similar experiments demonstrated to us that, as hypothesized by Brobeck, the central (ventromedial) area of the hypothalamus is concerned with satiety and acts as a brake on the constantly activated "feeding" areas. These experiments also show that it is on this "central" area that the organism indirectly acts to quantitatively determine food intake. In other words, what is "regulated" is not "hunger" but "satiety."

The answer to the second question, the manner in which this regulation operates, was first suggested by the following reasoning: appetite proceeds by fairly frequent partaking of food (meals). It appeared improbable that hypothalamic centers would be sensitive to decreases of the body content in fat and protein; these are proportionately enormous, representing as they do a great part of the body. During the interval between meals, their decrease would represent an insignificant proportion of the total. On the other hand, the body stores of carbohydrates are limited. In man, the liver content of glycogen after a meal is of the order of 75 grams, only 3000 calories worth. Glucose appears to be the essential, if not the only fuel of the central nervous system. It seemed legitimate to postulate that the "satiety centers" of the central hypothalamus might, in fact, be glucoreceptors, that is, they might be sensitive to blood glucose *in the measure that it could be ultilized.*

The limitations of space do not permit an elaborate description of the evidence used to verify this hypothesis. In a first series of experiments, with Dr. Margaret Bates, it was shown that in normal and diabetic animals, and in animals subjected to various hormonal treatments, decreased glucose availability or utilization correlated well with increased food intake. It was also shown that there was good correlation between decreased liver glycogen and feeding behavior. In a second experimental series, conducted with Dr. Van Itallie, we measured differences between the blood sugar level in arteries or capillaries and veins (Δ-glucose) in the forearm of a variety of human subjects.

It must be emphasized that this Δ-glucose is of no significance in itself; it is simply a general measure of glucose availability. The forearm is a more accessible area than the midbrain, if less directly representative of what goes on in the satiety centers. (There are circumstances, admittedly exceptional, where utilization does not vary in the same direction in both areas, for example, after administration of epinephrine.) Antecubital arteriovenous differences correlated well with subjective feelings of hunger when the rate of utilization fell.

These observations were confirmed and extended by Dr. Albert Stunkard of Cornell, who also reintroduced the use of the stomach balloon technic of Cannon and Carlson to record hunger contractions. Dr. Stunkard found that small arteriovenous glucose differences coincided generally with "hunger" gastric contractions and subjective feelings of hunger, while large differences accompanied satiety. Stunkard and Van Itallie have also shown that the pancreatic hormone, glucagon, which causes an elevation of the blood glucose level through glycogenolysis without any decrease in utilization, invariably eliminates gastric contractions and hunger feelings in man. In a particularly striking experiment, Stunkard studied a decorticate man in whom conscious influences were obviously eliminated and only the automatic mechanism still functioned. In this patient only food or the increased availability of glucose brought about by glucagon eliminated gastric hunger contractions. All other attempted treatments failed.

An even more direct indication in favor of a glucostatic intermediary for metabolic influences is yielded by the mode of action of the chemical goldthioglucose. This compound is made of a molecule of glucose linked to an atom of gold by a sulfur bridge. A single injection of goldthioglucose induces permanent overeating in mice, and, as a

result, produces gross obesity. The weight of the animals so treated can reach three times the normal. Goldthioglucose hyperphagic mice no longer increase their food intake in response to cold or enforced exercise. Dr. Normal Marshall and I were able to show that goldthioglucose causes selective destruction in the satiety areas (ventromedial) of the ventral hypothalamus. The effect could also be observed in rats. Furthermore, when gold was linked by a sulfur bridge to many compounds other than glucose, even though the over-all toxicity of the resulting substances might be the same as that of goldthioglucose, destruction of the satiety center and overeating did not follow. This was true even if the substituted compound was very similar to glucose, as in the case of goldthiosorbitol, or if it was one of the normal intermediaries in other pathways of metabolism, as in goldthiomalate, goldthiocaproic acid and goldthioglycerol. On the face of this evidence, it appeared legitimate to conclude that the satiety centers are indeed "glucoreceptors" and that goldthioglucose exerts its destructive effect because the gold is "dragged in" by glucose, for which the satiety cells have special affinity.

An important remaining problem is the mechanism whereby such a passage of glucose into the cells could be transformed into electrical (nervous) discharges. Such a mechanism is suggested by the fact that phosphate and potassium ions go into the cells at the same time as glucose. As shown by Larsson, through isotopic methods, the ventromedial areas of the hypothalamus appear to be particularly avid for glucose and phosphate when an animal has been fasted for several hours. Recently developed technics that permit the recording of nervous impulses directly in the hypothalamic centers show much promise for the analysis of the various factors: glucose utilization, temperature, salt concentrations, hormones, etc. which may also influence this area.

The problem of control of food intake quite obviously does not end at the hypothalamic level. Once hypothalamic impulses have been produced, they have to be integrated, interpreted and acted upon by the brain cortex. We are just beginning to explore some of the possible pathways and relays and some of the areas of the surface of the brain, the frontal lobes in particular, which may be concerned in this process. At the cortical level many other physiologic factors, such as temperature, dehydration, ionic imbalance etc., which are known to modify the electric activity of the surface of the brain, may inhibit or facilitate the feeding processes. And this of course is where conditioned reflexes, habits, and emotional associations also intervene. Hunger is one of the most powerful motivators of animal (and human) behavior. Conversely, in man, the partaking of food has acquired emotional and social meanings over and above its physiologic significance.

A stystem as complicated as the mechanism of the regulation of food intake can obviously go out of order in a number of places and for a number of reasons. A previous review, where an etiologic viewpoint was taken of the problem of obesity, described genetic, traumatic, and environmental factors leading to hyperphagia. More recently, in a review dealing with the pathogenesis of obesity, two general types of obesities were distinguished: "regulatory" obesities, where the primary impairment is in the central mechanisms, and "metabolic" obesities, where a metabolic disorder is primarily responsible. The case of limitations of an otherwise normal mechanism also has to be kept in mind; for example, we have shown that both in animals and in men, decreasing physicial activity below a certain level is no longer followed by a corresponding decrease in food intake, and obesity ensues. A beginning of analysis of the mode of action of certain drugs and of certain causes of anorexia—regulatory or metabolic—has also been undertaken. Certainly the field of regulation of food intake has progressed at least to the point where some of the questions become clear, even though the answers are still missing.

(*Since this article was written, Dr. S. D. Morrison and I have identified two small symmetrical areas of the subthalamus dorsal to Anand's feeding centers, the bilateral destruction of which causes both aphagia and adipsia, with neither secondary to the other. These areas appear to constitute a primitive, undifferentiated center controlling both eating and drinking, as during the suckling period.*)

BIBLIOGRAPHY

MAYER, J.: Genetic, traumatic and environmental factors in the etiology of obesity, Physiol. Rev. **33:** 472, 1953.

——: The physiologic basis of obesity and leanness. Nutrition Abstracts & Reviews, Part I, **25:** 597, 1955: Part II, **25:** 871, 1955.

——: Some advances in the study of the physiologic basis of obesity. Metabolism, *In press.*

A Brief for the Investigation of Human Ecology

By Lawrence E. Hinkle, Jr. and Harold G. Wolff*

The history of thought has been characterized by swings in interest from the whole to the part, and then to the whole again. From the middle of the 19th century until recently, the study of the parts and elements of science was pursued so intensively and so successfully that it almost consumed the energies and interest of creative minds; but with the advent of the 20th century, the need for unifying knowledge and for the study of relationships between parts was again voiced in many areas of thought and effort. The physicist's concept of the continuity of matter and energy, and the changes in the major styles of the plastic arts may be cited as examples. In medicine, the reaction against the extreme of partitioning of inquiry was expressed by J. B. S. Haldane in his now classic monograph on respiration: "Since the time of Hippocrates, the growth of scientific medicine . . . has been based on the study of the manner in which . . . the human body expresses itself in response to change in environment. . . . Only through this study can we recognize and interpret a disturbance in health." The study of man in the context of his environment has again become a suitable subject for science.

It is noteworthy that by preference naturalists and, more particularly, biologists, have long observed living creatures in their context, attempting to understand their goal-directed behavior, and doing so with delight and profit. This preferred point of view of the naturalists has been amusingly appraised by Suner, who says that, while physiologists have built their science by means of designed experiments, biologists have built theirs by observing the experiments of nature. Designed experiments divide phenomena into small sections of space and time. Naturalists, on the other hand, have felt free to observe the course of life on a larger scale.

The attitude of the naturalist is crystallized in the study of ecology, that branch of science which deals with the interrelations between organisms and their environment. The work of Darwin provided the impetus for the development of this discipline, which was introduced by the German biologist Ernst Haeckel in 1868 to describe his studies on plants. Those biologists who have taken the broad view of ecology have seen it as embracing the study of any of the pertinent features of living organisms in their natural habitat. The ecologist is properly concerned with any aspect of the environment to which the organism must adapt, and with any of the adaptive mechanisms which the organism utilizes in dealing with its environment. He therefore considers the physiologic and biochemical adaptive mechanisms within the organism and the anatomic structures upon which these are based, as well as the behavior of whole organisms and of the colonies and societies which these organisms develop. In a given instance, the ecologist does not attempt to study all of the variables, but only those which appear to be pertinent; but he is constantly aware that a multiplicity of factors are operating, and feels free to make use of any pertinent body of knowledge, tool or method. A number of biologists, among whom J. W. Bews has been outstanding, have suggested that the ecologic discipline and the concepts associated with it might profitably be applied to the study of mankind.

Among scientists who concern themselves with man, the sociologists primarily have utilized the concept of human ecology. This is perhaps because a major part of the environment to which man must adapt consists of the people about him and the society within which he lives. Human ecology as a subdiscipline of sociology has studied the relationship between man and his environment largely by studying the factors which influence the spatial distribution of population groups. Yet it is evident that we must depart from such a limited utilization of the concept of human ecology if we are to develop a better understanding of man. All of man's relation to his environment, his behavior, his health, and the development of his social organizations must be viewed in the light of his biologic background and makeup, the physical milieu in which he lives, the culture of the society of which he is a member, and the many-faceted position which he occupies in the social structure. Much of the illness from which men suffer is an aspect of their interaction with their environment.

Thus, scientists who are concerned with the study of man must encompass the facts necessary to an understanding of the relationship between man and his environment. To whatever extent they are pertinent to a given experimental problem, their studies must make use of the methods and knowledge of the physical and biologic sciences, or indeed of any other discipline useful in the study of the human organism, its internal mechanisms for adaptation, and the effects of these adaptations upon the organism; and they must also make use of the methods and knowledge of the social, political and geographical sciences and of any other disciplines useful

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in the study of human societies, and of the interaction of groups of men with their environment.

It should be emphasized that such an orientation does not impose upon the investigator a paralyzing pluralism or the necessity of an intensive investigation of all of the factors involved. Indeed, it does not even necessitate the concurrent operation of "a team" of workers with different disciplines, although it requires that such workers be called upon for specific information. Human ecology is, first of all, the study of man in his proper setting; secondly, it assumes in its practitioners an awareness of the operation of multiple factors, even though only a few most pertinent be selected for special study.

In short, the purpose of human ecology as a category of study is to further an awareness of the diversity of factors that operate in the study of man and his relation to his environment, to encourage investigators to draw upon any body of knowledge which is pertinent to the problem at hand, and to use those investigative tools which are most applicable. Its further purpose is to abolish arbitrary barriers between the "natural" sciences and the "social" sciences as they relate to the understanding of man, and to bring together scientists of various backgrounds, not simply to exchange views as representatives of different disciplines, but to work together in the study of the living man in his natural habitat.

CORRESPONDENCE

To the Editor:

"A crabbed age and youth cannot live together"
—Shakespeare

The American Federation for Clinical Research was founded as a stimulus to and an outlet for the young investigator. Its rapid growth in the short period of fifteen years attests to the void that it has filled. However, there is available clinical evidence that it is suffering some of the phenomena of metamorphosis that all young mens' societies display in their teens. One of these is a tendency to be wary of, and superior to, young upstarts. When the clubrooms become crowded with jostling and babbling unknowns, the oldsters complain that their favorite chairs are taken, and that their ideations do not receive the deference and ready acceptance that is their due.

In an address to the Federation two years ago ("On Building Walls," C.R.P. *2:* 156, 1954), Wolf pointed out to us the hazards inherent in the erection of barriers, and yet we have erected one in our Federation that at least superficially appears to belie the very purpose for which we were originally organized. Interestingly enough, that barrier was erected at the very meeting which was the occasion for Wolf's presidential address, and the barrier was in the form of a unanimous vote to abolish associate membership in the Federation.

Prior to that date any young physician interested in clinical investigation could become an associate member in our association for a small fee, could receive notices of and attend meetings, could there be exposed to the stimuli of contacts with a large number of individuals infected with various strains of the virus of research, and was thus able to evaluate the consequences of this affliction in its various degrees of severity. We have now taken steps to minimize the possibility of cross infections by this route. In order to qualify for membership a young man must now have participated in and finished a research task to the point of having his work published.

Correspondence with your officers indicates that this action was not proposed or taken without reason, but it is probably wise for the membership to have an opportunity to reexamine the reasons and to consider anew whether or not the action was the only solution to the problems, and further whether, if it was the only solution, our face was not spited at the cost of a nose.

It had been the practice in the past to advance associate members to full membership on submission of evidence, in the form of a reprint, that they had been active participants in research. Until this had been done, an associate could not nominate others to the group and he could not hold office. The mechanics set up by the secretary of the society apparently were not successful in preventing difficulties from arising. Members did not produce reprints and therefore tended to remain in associate status until such time as they turned out to have been illegally elected to office or attempted to nominate other members. Some apparently took it with bad grace when informed that they could not do so.

The advantages of encouraging the young seem obvious. The need for increasing numbers of young men in research is a pressing one. Dr. Christian wanted ". . . to stimulate among young men a persisting interest in investigation in clinical and allied medical sciences." The existing arrangement which insists on accomplishment prior to membership is paradoxical if our society still feels that his aims were correct ones. Can we not compromise? Would it be too expensive or too troublesome to resume the acceptance of associate members, perhaps with definite time limitations (two or three years) within which the associate could qualify for full membership or be automatically dropped or responsored? Can we not resign ourselves to the fact that to do this may necessitate an increase in the moiety which we must vote each year to the secretary's secretary? Better a bit more bookkeeping and epistolatory needling of associates who are remiss in advancing themselves than that we continue to exclude the seeds from which we ourselves have grown.

One of our presidents has recently pointed out the vital importance of maintaining the scientific spirit in medicine (Chapman, C. B., "On the Teaching of the Science of Medicine," C.R.P. *4:* 161, 1956.) One of the effective means of accomplishing this is to make every effort to include the physician in scientific bodies like ours in his formative years.

John H. Peters, M.D.
V.A. Hospital and the Dept. of Medicine, Emory University School of Medicine
Atlanta, Georgia

To the Editor:

It has long been the custom at large scientific meetings to provide each participant with an official badge bearing his or her name and academic affilia-

tion. While this practice may be somewhat cumbersome, it would have many advantages for the members of the American Federation for Clinical Research, both at national and regional meetings.

Our membership is large and composed chiefly of young investigators who are not yet well-known. We would become acquainted with one another more easily if we could learn a fellow-member's name simply by glancing at his or her badge rather than relying on a hastily mumbled introduction. We ask that a discussor identify himself by name and city during formal sessions; is not identification equally desirable during informal discussions outside the auditorium? Finally, such a means of identification would aid the investigator whose name is spelled in a way which cannot possibly be divined from its pronunciation.

If the members of the American Federation for Clinical Research desire them, appropriate badges could probably be provided for the next national meeting. Considering their usefulness to the participants, the cost of such badges would be relatively small.

Lawrence G. Raisz
SUNY College of Medicine, Syracuse
November 27, 1956

NOTICES

Rules Applying to Abstracts

1. Five copies must be submitted. Abstracts must be double-spaced and must not exceed 300 words.

2. No tables or graphs can be published.

3. Authors should refer to CLINICAL RESEARCH PROCEEDINGS, vol. I, p. 118 (September 1953) for pertinent suggestions on the form to be used in submitting research abstracts.

4. Each abstract must be accompanied by a covering letter giving the name, address, age and membership status of each of the authors and stating which author will present the paper. No one who has passed his forty-first birthday may present the paper.

5. One of the authors must be a member, or the paper must be introduced by a member.

6. Abstracts must be postmarked not later than February 25, 1957. Air mail should be used whenever delivery by regular mail cannot be accomplished within 36 hours.

Application for Membership

1. Young research workers are encouraged to apply for membership. It is unnecessary to await a member's invitation to join the AMERICAN FEDERATION FOR CLINICAL RESEARCH.

2. There is one requirement for regular membership: publication of a meritorious investigation in clinical medicine or allied sciences. This should not be a case report or an abstract.

3. An applicant must ask a member of the Federation who knows him to sign his application.

4. Interested individuals should write to Dr. William W. Stead, Secretary-Treasurer, AMERICAN FEDERATION FOR CLINICAL RESEARCH, V. A. Hospital, Minneapolis 17, Minnesota.

Professional Group on Medical Electronics

Physicians, biologists, physicists and biophysicists are invited to participate, through the "affiliate" plan, in the activities of the Professional Group on Medical Electronics (PGME) of the Institute of Radio Engineers, Inc. (IRE). PGME aims to bring together doctors, electronic engineers, biologists and biophysicists to discuss the use of electronic technics in problems of medicine and biology. The "affiliate" will not be an IRE member and therefore will pay only a reduced PGME "affiliate" fee. He will receive notices of all local and national PGME meetings, PGME newsletters and the PGME transactions, the official group publication.

For further information, write to Mr. L. G. Cumming, Technical Secretary, The Institute of Radio Engineers, Inc., One East 79th St., New York 21, N. Y.

New Research Tools

The information reported here is obtained from manufacturers. All inquiries concerning items listed should be addressed to New Research Tools Editor, % Grune & Stratton, Inc., 381 Fourth Avenue, New York 16, New York. Include the name(s) of the manufacturer(s).

✦ **An "autoanalyzer"** which can be used as a continuous sampling device for the automatic measurement of blood urea nitrogen, blood sugar and blood calcium, using 0.2 cc. of blood per minute. Methods for transaminase, acid and alkaline phosphatase and CO_2 determinations by the apparatus are being developed. The Technicon Company.

✦ **Specially impregnated discs** to make possible the rapid and accurate identification of group-A hemolytic streptococci on a blood or infusion agar plate. Scientific Products, Division of American Hospital Supply Corporation.

✦ **Multiple purpose hematocrit pipette** for drawing exactly 0.1 ml. capillary blood, which can be centrifuged while still in the pipette. In addition to the hematocrit, sedimentation rate, erythrocyte and leucocyte counts, Hb concentration and blood glucose can be determined on same sample of blood. Delmar Scientific Laboratories.

✦ Selection of **nine prepared culture media** in plastic petri dishes ready for immediate use packaged in sterile polyethylene bags. The units are disposable. Media now available are blood agar, chocolate agar, E.M.B. agar, MacConkey agar, SS agar, bismuth sulfite agar, Sabouraud dextrose agar, mycosel agar and Littmann oxgall agar. Hyland Laboratories.

✦ **High-sensitivity thermistor thermometers** with recently developed probes suitable for tissue implantation, measurement of cardiac or blood temperatures (by means of a catheter), subcutaneous temperatures (by means of a hypodermic needle), etc. Yellow Springs Instrument Co., Inc.

✦ **Improved segmental plethysmogragh** for determining blood volume changes per pulse in any segment of a limb. A meter indicates these changes, and output can also be connected to recording device. Increased sensitivity makes it possible to obtain pulse waves from limbs with very restricted arterial flow. Electro-Medical Engineering Co.

Société Belge de Recherches Cliniques

The letter quoted below in part was sent from Brussels on January 22:

. . . A Society after the pattern of the American Federation for Clinical Research has just been created in Belgium. Its name is 'Société Belge de Recherches Cliniques.' Dr. H. Tagnon, who is a Senior Member of the Federation, had the basic idea and stimulated us to create this new society. We would very much appreciate it if the A.F.C.R. would be willing to publish in its Proceedings the announcement of the birth of our Society.

. . . We would like to keep in touch as closely as possible with your society. May I suggest, for instance, that every member of the Federation, while in Belgium, be 'de jure' members of our society?

Dr. J. Brihaye
President

The Federation is honored to make this announcement. It is hoped that there will be close liaison between the two organizations and that members of the Federation will take advantage of their "de jure" membership in the Société Belge de Recherches Cliniques while in Belgium.

Lawrence E. Hinkle, Jr., M.D.

Members Lost to the National Office

It would be appreciated if each member would review the following list and notify Dr. William W. Stead (V. A. Hospital, Minneapolis 17, Minnesota) of the current address of anyone on the list with whom he is in contact:

	Former address
Dr. Frank K. Abbot	Lackland A. F. B. San Antonio, Tex.
Dr. Ronald D. T. Cape	7 St. Augustine's Rd., Edgbaston Birmingham, England
Dr. Michele Gerundo	Guam Memorial Hospital Guam, M. I.
Dr. John F. Gillespie	2222 Eye Street, N.W., Washington, D. C.
Dr. George B. Gordon	Fitzsimons Army Hospital Denver, Colo.
Dr. Marvin M. Hirsch	Dept. of Preventive Medicine U. of Illinois College of Medicine, Chicago, Ill.
Dr. Israeli A. Jaffe	562 West End Avenue New York City
Dr. Robert L. Johnson	Sheppard A. F. B. Wichita Falls, Tex.
Dr. Donald W. Rennie	Arctic Aeromedical Research Laboratory A. P. O. 731, Seattle, Wash.
Dr. Milton E. Rubini	Kings County Hospital Brooklyn, N. Y.
Dr. Michel M. B. Saint-Paul	9 Quai Turenne Nantes (L. Inf.) France
Dr. Ernest L. Sarason	608 E. Genesee St. Syracuse, N. Y.
Dr. Irvin Zeavin	4145 Garfield St. Lincoln, Neb.

Obituaries

The National Office has been notified of the death of the following members:

Dr. Robert Charr, Philadelphia, Pa.
Dr. Lawrence Greenman, Pittsburgh, Pa.
Dr. Malcolm McCord, Glendale, Ohio
Dr. Karl Singer, Chicago, Ill.
Dr. Herbert A. Weitzner, Berkeley, Calif.

Acknowledgment

It is the primary purpose of the American Federation for Clinical Research to provide an opportunity for young investigators in the medical sciences to take part in scientific meetings, and to present and publish the results of their work. In order to attain this goal it has been necessary to maintain the dues of the organization at a modest level, which does not place a burden upon men with residency or fellowship status. For many years, therefore, the Federation has financed its meetings and publications in part by the generous support of various companies which manufacture drugs, pharmaceuticals and scientific apparatus.

In recognition of the valuable assistance which these concerns have given to the Federation, the Council, at its Annual Meeting in May 1954, established the categories of Sponsor, Supporting Member and Contributing Member. The officers wish to take this opportunity to acknowledge the generous help given to the Federation by the following concerns in 1957.

Burroughs Wellcome & Co., Inc.	Sponsor
Lederle Laboratories Division, American Cyanamid Company	Sponsor
Sandoz Pharmaceuticals	Supporting Member
G. D. Searle & Co.	Supporting Member
Merck, Sharp & Dohme Research Laboratories	Supporting Member
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Lawrence E. Hinkle, Jr., *President*
Ivan L. Bennett, Jr., *Vice President*
William W. Stead, *Secretary-Treasurer*

PROGRAM

National Meeting

American Federation for Clinical Research

Sunday, May 5, 1957

Steel Pier Theater, Atlantic City, New Jersey

Dr. Lawrence E. Hinkle, Jr., Presiding

Presentations will be limited to ten minutes; five minutes will be allowed for discussion.

MORNING SESSION

9:00–9:15—Business Meeting

1. **The Effect of Progesterone on the Respiration of Patients with Emphysema and Hypercapnia**
   *John M. Tyler.** Lemuel Shattuck Hospital, Boston. (Introduced by *Thomas C. Chalmers*). *page 229*

2. **A New Method for Rapid Measurement of Hepatic Blood Flow and Portal Circulation Times Employing Radioactive Indicator Dilution Technics**
   *Stanley Reichman, Richard Gorlin, John Storaasli** and *William D. Davis, Jr.* U. S. Naval Hospital, Portsmouth, Va. *page 213*

3. **A Pressor Mechanism Associated with the Postpartum State**
   *Frank A. Finnerty, Jr. and Joachim H. Buchholz.** Georgetown University School of Medicine and District of Columbia General Hospital, Washington, D. C. *page 172*

4. **The Effect of Induced Cardiac Acceleration upon the Coronary Hemodynamics and Cardiac Metabolism of the Intact Anesthetized Dog**
   *G. M. Maxwell, C. A. Castillo,** *G. G. Rowe and D. H. White, Jr.** and *C. W. Crumpton.* University of Wisconsin School of Medicine, Madison. *page 176*

5. **Observations on the Mechanism and Treatment of Shock Following Myocardial Infarction**
   *R. W. Gunton, W. Paul** and *C. R. Woolf.** University of Toronto and Toronto General Hospital, Toronto. *page 164*

6. **Hemodynamic and Electrolyte Studies in Acute Glomerulonephritis**
   Yoshikazu Morita. Wayne State University College of Medicine, Detroit. *page 206*

7. **The Renal Red Cell Shunting Mechanism and the Control of Renal Resistance**
   Lawrence S. Lilienfield and John C. Rose. Georgetown University Medical Center, Washington, D. C. *page 203*

8. **Active Renal Regulation of Urea Excretion in Man**
   *Herschel V. Murdaugh, Jr. and Bodil Schmidt-Nielsen.** Departments of Medicine and Zoology, Duke University, Durham. *page 204*

9. **A Six-Year Follow-Up Study of Hereditary Interstitial Pyelonephritis**
   *G. T. Perkoff, Charles A. Nugent,** *Fayette E. Stephens** and *Frank H. Tyler.* University of Utah College of Medicine, Salt Lake City. *page 209*

10. **The Immunologic Behavior of *Escherichia coli* Endotoxin**
    *A. I. Braude, Jennie Siemienski** and *Robert Cade.* University of Texas, Southwestern Medical School, Dallas. *page 137*

11. **Relationship between Pyelonephritis and Bacterial Counts in Urine: Autopsy Study**
    *Richard A. MacDonald,** *Howard Levitin,** *G. Kenneth Mallory** and *Edward H. Kass.* Harvard Medical School, Boston University School of Medicine, and Thorndike Memorial Laboratory, Boston City Hospital, Boston. *page 207*

12. **Primary Hyperaldosteronism, Long-standing Potassium Depletion, and Pyelonephritis**
    Robert C. Muehrcke and Malcolm D. Milne. Presbyterian Hospital, Chicago, and Postgraduate Medical School of London. *page 190*

AFTERNOON SESSION

1:45–2:00—Business Meeting
2:00—Presidential Address

13. **Conversion of "Indirect" to "Direct" Reacting Bilirubin by Human and Rat Liver in vitro and Studies of a Defect in Bilirubin Conjugation in Constitutional Hepatic Dysfunction (Gilbert's Disease)**
    *Irwin M. Arias and Irving M. London.** Albert Einstein College of Medicine, New York. *page 213*

* By invitation

SUBSECTION PROGRAMS

Subsection on Cardiovascular Disease

Carolina Room, Haddon Hall, 8 p.m., Sunday, May 5, 1957

Dr. Daniel S. Lukas, Chairman, Presiding

1. **Intracardiac Phonocardiography in Man**
   David H. Lewis, George W. Deitz, Ali Ertugrul,* John D. Wallace* and James R. Brown, Jr.** Philadelphia General Hospital, Philadelphia, and U. S. Naval Air Development Center, Johnsville, Pa. *page 166*
2. **An Appraisal of the Korner-Shillingford Method for Measurement of Valvular Regurgitation**
   Paul Novack, Robert C. Schlant, Florence W. Haynes,* Arthur O. Phinney, Jr.* and Lewis Dexter.* Harvard Medical School and Peter Bent Brigham Hospital, Boston. *page 165*
3. **Measurement of Valvular Insufficiency by an Indicator-Dilution Method**
   William R. Milnor. Johns Hopkins Hospital, Baltimore. *page 165*
4. **The Oxygen Cost of Breathing in Dyspneic Subjects as Studied in Normal Pregnant Women**
   Richard A. Bader, Mortimer E. Bader and David J. Rose. Mount Sinai Hospital, New York. *page 226*
5. **The Effect of Serotonin upon Systemic Small and Large Vessel Resistances**
   F. J. Haddy, M. Fleishman, D. A. Emanuel* and J. B. Scott.** U. S. Army Medical Research Laboratory, Ft. Knox, Ky. *page 174*
6. **A Correlation of Electrocardiographic and Hemodynamic Changes Following Closure of Atrial Septal Defects**
   Leonard S. Dreifus, Sheldon Bender, Janet Dickens,* Daniel F. Downing and Harry Goldberg.* Hahnemann Medical College and Hospital, and the Bailey Thoracic Clinic, Philadelphia. *page 169*

Subsection on Gastroenterology

Viking Room, Haddon Hall, 8 p.m., Sunday, May 5, 1957

Dr. Albert I. Mendeloff, Chairman, Presiding

GASTRIC MOTILITY AND SECRETION

1. **Simultaneous Multi-Electrode Recording of Gastric Potential**
   George M. Katz, Henry Colcher and Edmund N. Goodman.** Columbia University College of Physicians and Surgeons, New York. *page 195*
2. **Diurnal Variation of Uropepsinogen and Its Relation to Adrenal Function**
   J. A. Vennes, R. P. Doe, D. Gleason and E. B. Flink.* University of Minnesota, Minneapolis. *page 197*
3. **The Physiologic and Clinical Limitations of Blood Acid Protease (Blood Pepsin)**
   H. M. Spiro, E. Friedman and I. J. Poliner.* Yale University School of Medicine, New Haven. *page 196*

General Discussion

PANCREATITIS AND HYPERLIPEMIA

4. **Hyperlipemia and Pancreatitis: in Man and in Experimental Animals**
   Chun I. Wang, Fiorenzo Paronetto and David Adlersberg.* Mount Sinai Hospital, New York. *page 197*
5. **Serum Lipids and Lipid Enzymes in Acute Pancreatitis with Lipemia**
   Harold H. Orvis and John M. Evans.* George Washington University School of Medicine, Washington, D. C. *page 197*

General Discussion

HEPATIC FUNCTION

6. **Clinical Studies with Radioiodine Rose Bengal Dye**
   James A. Wood and Donald R. Korst.* University of Michigan and Veterans Administration Hospital, Ann Arbor. *page 210*
7. **The Significance of Alterations in Serum Enzymes in the Differential Diagnosis of Jaundice**
   Felix Wróblewski. Memorial Center, New York. *page 212*

General Discussion

Subsection on General Metabolism

Rutland Room, Haddon Hall, 8 p.m., Sunday, May 5, 1957

Dr. A. B. Falcone, Chairman, Presiding

1. **The Metabolic Conversion in Man of 21-Carbon and 19-Carbon-1,4-Diene Steroids to 17-Ketosteroids**
   Maurice M. Pechet and Jane Claffey.** Massachusetts General Hospital and Harvard Medical School, Boston. (Introduced by *Perry J. Culver.*) *page 191*
2. **Base Binding Property of Serum Proteins for Calcium**
   Ananda S. Prasad and Edmund B. Flink. Veterans Administration Hospital, Minneapolis. *page 157*
3. **Dialyzable and Nondialyzable Components of Serum which Promote Sulfate Uptake by Cartilage from Hypophysectomized Rats in Vitro**
   William D. Salmon, Jr. and William H. Daughaday.* Washington University School of Medicine, St. Louis. *page 186*
4. **Inhibition of Cholesterol Absorption by Wool Fat Sterols**
   Maurice M. Best and Charles H. Duncan. University of Louisville School of Medicine, Louisville. *page 182*
5. **Effect of a Synthetic Steroid on Serum Lipids and on Serum Protein-Bound Iodine**
   John H. Peters, A. Henry Randall and Mary G. Bell.** Emory University School of Medicine and Veterans Administration Hospital, Atlanta. *page 183*
6. **A New Diagnostic Test for Early Diabetes Mellitus**
   Roger H. Unger and Leonard L. Madison.* University of Texas Southwestern Medical School and Veterans Administration Hospital, Dallas. *page 187*

Subsection on Medical Education

West Room, Haddon Hall, 8 p.m., Sunday, May 5, 1957

Dr. Henry D. McIntosh, Chairman, Presiding

1. **The One-way Screen as an Adjunct to Clinical Teaching**
   Clinton G. Weiman and George G. Reader.* New York Hospital-Cornell Medical Center, New York. *page 179*
2. **A Method for Evaluating Student-Patient Interviews**
   Guy Hollifield, C. T. Rousell, A. J. Bachrach* and E. G. Pattishall.** University of Virginia School of Medicine, Charlottesville. *page 178*
3. **The Psychotherapy Study Group: A Method of Postgraduate Education in Psychotherapeutic Aspects of Medical Practice**
   *Albert Stunkard and Peter Janulis.** New York Hospital-Cornell Medical Center, New York. *page 177*
4. **The Professional Educator: Enemy or Friend?**
   George E. Miller. University of Buffalo School of Medicine, Buffalo. *page 178*

Subsection on Renal Disease and Electrolyte Metabolism

Music Room, Haddon Hall, 8 p.m., Sunday, May 5, 1957

Dr. Jack Orloff, Chairman, Presiding

1. **Dialysance of Bromide from Blood and Spinal Fluid**
   George E. Schreiner and Leonard B. Berman. Georgetown University Medical Center, Washington, D. C. *page 233*
2. **A Mechanism by Which Altered Distribution of Blood Volume Affects Renal Function**
   Herbert O. Sieker and Herschel V. Murdaugh, Jr.* Duke University School of Medicine and Veterans Administration Hospital, Durham. *page 204*
3. **The Degree of Granulation of the Renal Juxtaglomerular Apparatus in Relation to Hypertension and Sodium Intake**
   Louis Tobian, Janet Thompson and Robert Twedt.** University of Minnesota School of Medicine, Minneapolis. *page 234*
4. **Influence of Potassium Deficiency on Response to an Acidifying Salt**
   M. E. Rubini, W. B. Blythe, E. G. Herndon and W. H. Meroney.* Walter Reed Army Institute of Research, Washington, D. C. *page 193*
5. **The Role of Nonhormonal Factors in the Impaired Water Diuresis Associated with Addison's Disease and Anterior Pituitary Insufficiency**
   Charles R. Kleeman and Morton H. Maxwell. University of California Medical Center and Veterans Administration Center, Los Angeles. *page 191*

Advance Research Reports Submitted to the Annual

National Meeting

of the

American Federation for Clinical Research

Steel Pier Theater, Atlantic City, New Jersey · Sunday, May 5, 1957

ALLERGY

The Release of Serotonin and Histamine During Anaphylaxis in the Rabbit

By *T. P. Waalkes, H. Weissbach, J. Bozicevich and S. Udenfriend.* National Heart Institute, and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

Histamine is released during anaphylaxis both in vitro and in vivo. Recent work has shown that 5-hydroxy-tryptamine (serotonin) is also released when antibody and antigen are added to normal rabbit platelets suspended in plasma. In this study, when the antigen was incubated with whole blood from a rabbit sensitized to this antigen, both serotonin and histamine were liberated from the platelets. Intravenous injection of the antigen into sensitized rabbits caused an immediate rise in plasma serotonin and histamine. The maximum serotonin level was reached in one minute, and returned to normal in two minutes. In comparison, plasma histamine levels rose to higher maximum values and returned to normal more slowly. However, whole blood serotonin and histamine levels fell during anaphylaxis; minimum values were found within four minutes, followed by a gradual rise. This drop in serotonin and histamine paralleled the fall in platelet count.

Serotonin and histamine are also found in tissues. Whether or not these amines are released from platelets and/or tissues during anaphylaxis is important. Reserpine (5 mg./Kg.) liberates both serotonin and histamine from platelets, but only serotonin from tissues. By using less reserpine (0.1 mg./Kg.), platelets can be depleted of serotonin and histamine without lowering the total serotonin content of the tissues. In rabbits treated with reserpine (0.1 mg./Kg.), plasma levels for serotonin did not rise during anaphylaxis, whereas the levels for histamine were increased. These findings suggest that most of the serotonin in plasma during anaphylaxis is released from platelets while the histamine is released from platelets and tissues.

Tissue studies after anaphylaxis revealed changes in the serotonin and histamine content which may be of importance in the manifestations and outcome of this phenomenon.

The Immunologic Behavior of Escherichia Coli Endotoxin

By *A. I. Braude, Jennie Siemienski and Robert Cade.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

The mechanism of action of toxic non-protein lipopolysaccharides of gram-negative bacteria is unknown. Because these endotoxins possess antigenic specificity, it is possible that their physiologic effects result from immunologic reactions with antibodies. The immunologic behavior of *E. coli* endotoxin was therefore investigated in normal persons and animals and compared to those infected

with *E. coli*. For this purpose, circulating antibody was measured by precipitin and complement fixation in the presence of 0.5 mg. endotoxin, and tissue antibody by intradermal reaction to 0.1 μg. endotoxin.

All of 45 normal adults displayed local reactions characterized by erythema and painful induration beginning in 2 hours, reaching a maximum in 12 hours and completely subsiding by 48 hours. Six experienced transient lymphangiitis, three arthralgia, and 2 fever. This universal dermal reactivity was also found in 44 patients with variable diseases including 8 with *E. coli* pyelonephritis and one with agammaglobulinemia. Negative reactions were found only in 6 of 14 children under four. The dermal reactivity was accompanied by absence of circulating antibodies in all persons tested although the antigen gave a strong complement fixation with sera of tolerant rabbits.

Absence of antibody was also observed in sera of normal animals. *E. coli* pyelonephritis was therefore established in rats to determine whether recovery from infection at 8 weeks is accompanied by antibody production. At 3 weeks, weak complement-fixation was observed in two sera and at 8 weeks no fixation was present despite extensive healed pyelonephritis in all animals.

These results indicate that dermal reactivity to *E. coli* lipopolysaccharides (1) develops in all children and persists throughout life; (2) is unaccompanied by circulating antibodies even after infection; (3) may be associated with systemic reactions resembling those during *E. coli* infections; and (4) unlike tuberculin sensitivity, is not associated with resistance to infection.

BLOOD

Simultaneous Inheritance of Three Adult Hemoglobins Determined at Two Genetic Loci

By *John V. Torbert, Jr. and Ernest W. Smith.* Department of Medicine, The Johns Hopkins University, Baltimore.

Four members of one Negro family had three electrophoretically distinct hemoglobins. Studies of the kindred demonstrated that one of the abnormal hemoglobins is determined at a genetic locus other than that concerned with the production of hemoglobins A, C and S.

Studies were done on 15 members of the kindred. The first generation female, two second generation females, and a third generation child had all three hemoglobins, hemoglobin A, hemoglobin S, and "fast" hemoglobin. Another second generation female had sickle trait. The remaining 8 individuals were of the third generation, and of these, 4 had sickle trait and 4, including the propositus, had "fast" and A hemoglobins. The only reasonable explanation for this segregation of hemoglobins is that the fast component is determined at a locus other than that responsible for hemoglobins A and S. The expected offspring with only hemoglobin A were not encountered.

Using filter paper electrophoresis at pH 8.6, the relative mobility of the fast component was significantly greater than normal. Also, the proportion of "fast" hemoglobin was always less than A or S. Hematologic studies including red cell indices and morphology, white cell and reticulocyte counts, serum bilirubin, fetal hemoglobin, and osmotic and mechanical fragility of fresh and incubated erythrocytes were within normal limits. Sickle cell preparations were positive in individuals with hemoglobin S.

Except for this family, the "fast" hemoglobin has not been encountered among 6,000 Negroes selected at random from The Johns Hopkins Hospital. Its identification as one of the previously reported hemoglobins or as a new genetic abnormality has not yet been accomplished.

Arterial Oxygen Desaturation in Patients with S-Type Hemoglobin Patterns

By *Brial J. Sproule, E. Richard Halden and William F. Miller.* Cardiopulmonary Laboratory of the Department of Medicine, University of Texas Southwestern Medical School, Dallas. (Aided by a U. S. Public Health Service grant.)

During studies of the effects of low oxygen breathing on the in vivo sickling phenomenon, exaggerated arterial oxygen desaturation was frequently noted.

To investigate the frequency and mechanism of this finding, blood gas studies at different levels of inspired oxygen were performed on 19 patients with various S-type hemoglobin patterns. Reduced arterial oxygenation ($SaO_2 < 95\%$; $PaO_2 < 80$ mm. Hg) occurred in 15 of the 19 patients, with an average SaO_2 of 89.2%, PaO_2 63.4 mm. Hg, and alveolar-arterial pO_2 gradient 36.8 mm. Hg on ambient air breathing. Desaturation was not related to the magnitude of anemia, to the specific electrophoretic pattern, or to the sickling phenomenon.

On low oxygen breathing transfer gradients averaged 11.1 mm. Hg and exceeded 15 mm. in only 3 instances, suggesting that diffusion disturbances are not likely to be a major cause of the increased ambient air gradients.

Utilizing a new polarographic technic, A-a gradients while breathing 100% oxygen averaged

26 mm. Hg in the normally saturated subjects; in the desaturated group, they ranged from 79 to 457 mm. with an average of 203 mm. This is construed as evidence of central venoarterial admixture.

Cardiac catheterization was performed on 7 of these patients, all with roentgenographic evidence of cardiomegaly. T-1824 dye curves showed no evidence of intracardiac venoarterial admixture. Cardiac indexes were elevated in the hypoxic patients (5.55 L./sq. m./min.) and pulmonary vascular resistance was within normal limits in all. Dynamic evidence of right ventricular failure was present in one patient.

All of the patients had restrictive ventilatory disturbances evidenced by reduced vital capacities averaging 65% of the predicted value.

The work establishes the presence of right-to-left shunting in many patients possessing S-type hemoglobin. The site of such shunting is probably intrapulmonary rather than intracardiac.

A Possible Diagnostic Test For Thalassemia

By *Aaron M. Josephson, Merle S. Masri, David Dworkin, Lily Singer and Karl Singer.* Department of Hematologic Research, Medical Research Institute, Michael Reese Hospital, Chicago.

The diagnosis of thalassemia is frequently made by exclusion. Chronic iron deficiency anemia is often a differential diagnostic problem. Hemolytic anemias in children may also present etiologic diagnostic difficulties. In 1955, Kunkel and Wallenius applied the technic of starch block electrophoresis (pH 8.6) to the separation of hemolysates. They found that normal adult hemoglobin separated into three components: A_1, the main component; A_2, the slow component; and A_3, the fast component. They also found the A_2 component to be increased in patients with the thalassemia trait. Using this method a study of 20 genetically and hematologically proven thalassemic patients was performed. 25 normal individuals, 12 patients with iron deficiency anemia due to bleeding, and a number of other patients with hematologic disorders other than thalassemia were also studied. The amount of A_2 was found to be consistently elevated above the normal value only in those patients who had the thalassemic syndrome. There was no correlation of the amount of A_2 with the amount of fetal hemoglobin or with the severity of the disease. In all cases, simultaneous Tiselius electrophoresis was performed. With this technic at pH 6.5 a faster moving component was consistently seen which seems to be quantitatively identical with the A_2 fraction found on starch.

Studies On The Effectiveness Of An Iron Chelate In Hemoglobin Regeneration

By *Marvin J. Seven and Ralph E. Peterson.* National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

Studies were made to determine the effectiveness of an iron chelate in the regeneration of hemoglobin in two subjects made iron-deficient by repeated phlebotomies. The Fe^{59}-tagged ferric chelate of N-hydroxyethylethylenediamine triacetic acid (Versenol) was administered intramuscularly to one subject. Radioactivity determinations showed a rapid disappearance from the injection site, high plasma levels up to 2 hours, and incorporation into circulating red blood cells at 12–24 hours. 8.8% was excreted in the urine within 24 hours, and fecal output was 0.1% over 4 days. Ninety per cent of the injected radioactivity was found in circulating red blood cells after 12 days. Serum iron levels following intramuscular injection of 40 mg. nonradioactive iron showed that breakdown of the chelate was rapid. Serum iron increased from 22 to 71 μg. % in 30 minutes, completely saturating the serum iron-binding protein at 4 to 8 hours. The method used did not determine serum iron in the chelated form. Therapeutic doses of iron were given, sufficient to raise the hemoglobin level from 9.4 to 12.7 Gm. %.

The Fe^{59}-tagged chelate was given orally and intraduodenally to the second subject. Absorption from the gastrointestinal tract was incomplete, with 3.4% appearing in the circulating red blood cells after oral administration and 2.4% after intraduodenal administration. Most of the radioactivity appeared in the feces over a 4-day period.

Our data suggest a new clinical application for specific chelates. Apparently these highly stable complexes may be broken down within the body to release a specific metal ion for utilization in a metal deficiency state. The intramuscular route may be an effective means of parenteral iron administration. Absorption of iron from the GI tract was not facilitated by the iron chelate complex.

Treatment of Hemochromatosis by Energetic Phlebotomy

By *William H. Crosby and Thomas W. Sheehy.* Walter Reed Army Medical Center, Washington, D. C.

A 48-year-old Army officer with hemochromatosis was phlebotomized to remove excess iron from his body as rapidly as possible. By means of frequent bleedings the hemoglobin concentration of his blood was maintained at 10 to 12 Gm. in order to stimulate hemoglobin production and mobilize iron from the storage depots. After 11 months the iron stores were exhausted. During this time 55 L. of blood were removed which contained 6 Kg. of hemoglobin and 20 Gm. of iron. The first 15 Gm. of iron were readily mobilized at rates as high as 130 mg./day. Hemoglobin production at this time was almost 40 Gm./day or 6 to 7 times the normal rate. Average

life span of the red cells was 18 days. The last 5 Gm. of iron came mostly from the liver, slowly at a rate of about 20 mg./day. After the iron stores were depleted the patient was anemic and he reconstituted his red cell volume over a period of 3 months. Most of the iron for the increase of hemoglobin came from his diet at the rate of about 6 mg./day. This is a high figure, the normal rate of absorption being about 1 mg./day. This high rate of absorption may represent the combined effects of anemia, which is supposed to increase the avidity of the intestine for iron, and of hemochromatosis, where the essential defect is an abnormal facility of the bowel for the absorption of iron.

During the course of treatment the patient's diabetes was considerably improved. He remained on duty except for the first few weeks.

The Relationship of the Red Cell Water and Mineral Content to Plasma Composition: Evidence of Cell Potassium Depletion in Chronic Adrenal Insufficiency, Renal Failure and Laennec's Cirrhosis

By *H. G. Keitel and H. Jones.* National Institutes of Health, Bethesda, the Massachusetts General Hospital, Boston, and the Department of Pediatrics, Harvard Medical School, Boston.

The red cell content (quantity/Kg. cell solids) of water, sodium, potassium and chloride was compared to the plasma composition with respect to these substances in 103 patients with various medical, surgical and metabolic disorders. The data were evaluated according to the osmotic concepts developed earlier by Van Slyke, Wu and McLean, and by Darrow and Yannet. The sodium plus potassium plus chloride *concentration* (quantity/Kg. water) (Na^+K^+Cl) of red cells was directly correlated to the plasma concentration of these electrolytes ($Y = 17.7 + .8034\ x$; $P = 0.01$). However, the relationship of the sodium plus potassium (Na^+K) concentration alone, in the two fluid phases, was less significant ($Y = 79.5 + 0.467\ x$; $P = 0.05$), many points deviating considerably from the average relationship. The red cell *content* of sodium, potassium, or water could not be predicted from plasma composition with any degree of confidence.

A reduced red cell potassium content was found in many patients who had conditions that have been reported to be associated with generalized cell potassium deficiency, including diabetic acidosis, gastric alkalosis and "base losing" nephritis. Both reduced and elevated plasma potassium concentrations were found in these patients. An unexpected finding was the occurrence of reduced red cell potassium *content* in 4 patients with adrenal insufficiency, 7 of 13 patients with renal failure and hyperkalemia, and 3 of 4 patients with Laennec's cirrhosis. The red cell potassium content of the patients with adrenal insufficiency returned to normal following treatment with DOCA, sodium chloride, cortisone and diet *ad lib.*

Potassium depletion of red cells has been found only in diseases reported to be associated with total body cell potassium deficits in excess of nitrogen—as determined by the balance method. The present data, like those of Darrow (1944), Burrows, and Talbot and Cook, suggest that in adrenal insufficiency, cell potassium accumulation is not always found, notwithstanding the presence of hyperkalemia.

The Effect of Nucleosides on Osmotic Resistance of Mammalian Erythrocytes in Relation to the Age of the Cells

By *Ernst R. Jaffé, Grace A. Vanderhoff, Bertram A. Lowy and Irving M. London.* Department of Medicine, Albert Einstein College of Medicine, New York.

In studies on the relationship of metabolic activity of the erythrocyte to its structural integrity, the resistance of fresh human erythrocytes to osmotic stress has been found to be enhanced by incubation of the erythrocytes with various purine ribosides. The following experiments were designed to determine the influence of the age of the mammalian erythrocyte upon this effect of purine ribosides.

Whole blood from a normal adult male was collected in acid-citrate dextrose solution and stored at 4° C. At intervals during the subsequent 168 days, aliquots of the stored erythrocytes were washed with isotonic sodium phosphate buffer, pH 7.3, and incubated in the buffer with and without nucleosides or glucose. After incubation the osmotic fragility was determined. The effect of adenosine or inosine in enhancing resistance to osmotic lysis was most marked initially and declined progressively; after 168 days no effect was demonstrable. The protective effects of glucose and deoxyadenosine were minimal.

Adult male rabbits received glycine-2-C^{14} for labeling of newly formed erythrocytes. At intervals of 4, 8, 13, 46, and 60 days after injection of the labeled glycine, one of the rabbits was exsanguinated and the washed erythrocytes were incubated in isotonic sodium phosphate buffer, pH 7.3, with and without added nucleosides. After exposure to hypotonic phosphate buffer solutions, the extent of hemolysis and the radioactivity of the hemin isolated from the hemoglobin of the lysed cells were determined. It was found that young rabbit erythrocytes are more resistant to osmotic stress than older cells and that the effect of inosine in enhancing resistance to osmotic lysis is much more pronounced in young than in old erythrocytes.

The finding that the ability of purine ribosides to enhance osmotic resistance is more pronounced in young than in old erythrocytes is pertinent to the

problems of erythrocyte preservation and of the mechanism of aging of erythrocytes in vivo.

Adsorption of C^{14} Dextran to Human Blood Platelets and Red Blood Cells

By *S. Rothman, E. Adelson, A. Schwebel and R. D. Langdell.* National Bureau of Standards and Walter Reed Army Medical Center, Washington, D. C.

Many patients develop a hemostatic defect after infusions with dextran. Studies of the mechanism of this defect have pointed to the platelet as the cause. Since much of the platelet function is dependent on surface characteristics, this study was devised to demonstrate whether or not dextran coats the platelet surface.

A C^{14}-carboxy labeled dextran fraction (number—average molecular weight 90,600; 150 μc./Gm.) was incubated with whole blood drawn by the two syringe technic with siliconized glassware and sequestren anticoagulant for eight hours. The red cells and platelets were each separated by centrifugation and counted visually using a phase microscope. Each component was washed six times with a plasma solution of the cold dextran fraction to remove unadsorbed C^{14} dextran and then lyophilized. The platelets and red cells were each oxidized by the wet combustion technic and the carbon dioxide collected in an ionization chamber. The radioactivity of each sample was determined with a vibrating reed electrometer.

On the basis of the measured radioactivities and the predetermined numbers of platelets and red cells, it was possible to calculate the amount of dextran associated with each unit component. Whole blood from each of three patients was used; approximately 7×10^3 molecules of dextran were detected per platelet and 6×10^3 dextran molecules per red cell.

Erythropoietic Function in Uremic Rabbits

By *Allan J. Erslev.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Service, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

A moderate or often severe anemia is usually present in patients with chronic renal disease. The anemia is undoubtedly associated with a shorter than normal life span of red cells. However, the most important pathogenic mechanism appears to be a failure of the bone marrow to compensate for this hemolysis and so to maintain a relatively normal hemoglobin concentration.

Recently, it has been proposed that normal erythropoietic function is controlled, partly or wholly, by the level in the blood stream of an erythropoietic factor and that this level in turn is controlled by the tension of oxygen in a specific extramedullary site.

With this hypothesis in mind a study was made of the pathogenesis of the diminished red cell production in the anemia of uremia. Normal and uremic rabbits were exposed to a standardized hemorrhage and their erythropoietic responses were evaluated by means of reticulocyte counts, bone marrow examinations and bioassay for erythropoietic serum factor.

1. Rabbits made uremic by means of bilateral nephrectomy, ligation of the ureters or section of the ureters failed to respond to a single bleeding of 17 to 18 ml./Kg. with the usual increase in reticulocytes and bone marrow normoblasts.

2. Serum from rabbits rendered both uremic and anemic did not elicit a reticulocytosis when bioassayed in normal rabbits.

3. Serum from uremic rabbits did not inactivate the erythropoietic activity of serum from anemic but otherwise normal rabbits.

4. Uremic rabbits failed to respond with a reticulocytosis to the infusion of serum from anemic but otherwise normal rabbits.

These findings indicate that the anemia of uremic rabbits is unrelated to the presence or absence of normal kidney tissue, and is associated with both a decreased production of erythropoietic factor and a decreased erythropoietic response to this factor.

Relationship of Acute Liver Damage, Plasma Erythropoietic Factor and Erythron Response in Rats

By *T. C. Prentice and E. A. Mirand.* Roswell Memorial Park Institute, Buffalo, N. Y.

Recently the liver has been the focus of increased attention concerning its role in the mechanisms that control plasma erythropoietin levels. The present study is a further effort to clarify this role.

During the past year repeated attempts have been made to demonstrate significant amounts of plasma erythropoietin in the plasma of normal Sprague-Dawley rats placed in a low oxygen atmosphere (8–12%) for 48 hours. Uniformly negative results were achieved. Acute liver damage was then produced in these animals with CCl_4 prior to placement in the chamber. Contrary to the normals, these animals possessed significant amounts of erythropoietic factor in their plasma after removal from the chamber.

Although the animals with acute liver damage subjected to hypoxia possessed significantly increased amounts of EPF in their plasma, as evidenced by increased RBC Fe^{59} uptake in hypophysectomized recipients of their plasma, they themselves did not respond to erythropoietic stimuli (hypoxia, phenylhydrazine-anemic plasma) any

more efficiently, and possibly a little less, than the normal controls. Response was measured by Fe^{59} uptake in RBC's, reticulocyte counts and nucleated RBC's in marrow.

It therefore appears that although liver damage makes possible greater delivery of EPF to plasma under erythropoietic stimulus, effective erythropoietic response is not improved by this procedure. This parallels some of the anemias in human beings where marked increases of EPF are found in the plasma but no sign of response in the patient is seen.

The conclusion is either (1) that some mechanism is blocking the effectiveness of EPF under these conditions, or (2) that the so-called EPF is an incidental by-product in the plasma and has no significant place in the mechanisms normally controlling RBC production.

Assay of Erythropoietic Factor(s) Using Radioiron Uptake in the Nitrogen Mustard Treated Rat

By *Donald R. Korst and Frank H. Bethell.* Radioisotope Service, V. A. Hospital and The Thomas Henry Simpson Memorial Institute for Medical Research, Ann Arbor, Michigan.

A method of measuring erythrocyte incorporation of Fe^{59} in rats over a three day period has been described. Depression of iron uptake in erythrocytes occurs after I.V. injection of nitrogen mustard (HN_2 0.2 mg./Kg.), if the tracer is given within 48 hours of HN_2 administration. After this interval, the uptake is accelerated, probably due to regeneration of marrow. Degree of depression of the radioiron uptake is progressive with larger doses of HN_2. 0.4 to 0.6 mg./Kg. result in 5 ± 2% utilization of Fe^{59} at 18 hours, 6 ± 3% at 24 hours, and 5 to 10 ± 4% at 42 hours. The normal rat not given HN_2 has an uptake of about 36 ± 10% at 24 hours and 51 ± 9% at 48 hours.

Plasma from phenylhydrazine anemic rabbits (PAP) was given intraperitoneally to rats in doses equal to 2% of their body weight at 24 hours and 17 hours prior to, and at the time of, Fe^{59} injection. Blood specimens were then drawn from the tail vein at 18, 24 and 42 hours. Hemoglobin and radioactivity were measured on each sample. The experiments occupied 4 days and normal rabbit or human plasma was given to control groups for each determination. Animals weighed 130–160 Gm. and there were 4 in each group. Frozen stored whole PAP induces an Fe^{59} utilization of 24 ± 4% at 18 hours, 33 ± 3% at 24 hours, and 50 ± 3% at 42 hours when there is an interval of 48 hours between HN_2 and Fe^{59} injection. A portion of the same PAP boiled three times for 15 min. and filtered and reconstituted to original volume gave the same results as the normal rabbit plasma (NR) control (5 ± 2% at 18 hr., 6 ± 2% at 24 hr., and 25 ± 3% at 42 hr.).

Plasma or serum from patients with polycythemia or various types of anemia is being tested. Preliminary results suggest that there may be at least two erythropoietic factors. One factor is thermolabile and is present in fresh plasma. It increases Fe^{59} utilization. Another is heat stable, which is present in boiled plasma extracts and increases the rate of normoblast division.

Differences in Electrolyte Composition and Potassium Exchange Associated with in vivo Aging of Human Erythrocytes

By *E. Raymond Borun.* Veterans Administration Center and the Department of Medicine, University of California Medical Center, Los Angeles.

Previously reported data demonstrated that the top, middle, and bottom layers of centrifuged erythrocyte specimens represent young, intermediate, and old mean cell ages, respectively. Chemical and metabolic differences between these layers can therefore be related to mean cell age.

Heparinized blood specimens from subjects without hematologic or electrolyte disturbances were centrifuged in plastic tubes and placed in a liquid freezing mixture. The portion of the tube containing frozen erythrocytes was cut into four equal layers, and thawed material from each layer was used for chemical and isotope analysis. Trapped plasma was determined with radioiodinated serum albumin. K^{42} uptake was determined in the erythrocyte layers of specimens incubated at 37° C., with and without a preceding 24 hour period of storage at 4° C.

Data from 12 specimens indicate that old erythrocytes in the bottom layer contain significantly less water, potassium, chloride, and bicarbonate than younger cells in the upper layers. These results are consistent with data published by Keitel. The old erythrocytes contain a lower concentration of potassium and slightly higher concentration of sodium per liter of cell water (mean difference ± S.E. = 10 ± 1 and 3 ± 0.5 mEq./L., respectively). A smaller difference between total determined cation and anion in the old erythrocytes (20 ± 4.5 mEq./Kg. dry weight) suggests that aging is associated with a decrease in organic anion.

The old erythrocytes show a higher K^{42} specific activity and a slightly more rapid rate of potassium exchange per liter. The presence of a more rapidly exchanging fraction of potassium in the dense, older erythrocytes confirms the suggestion of others that the erythrocyte potassium "compartment" is not homogeneous. Accentuation of the difference in potassium exchange by prior storage of erythrocytes at 4° C. implies a metabolic factor in addition to the probable effect of differences in the ratio of surface area to volume.

The Use of Diisopropylfluorophosphate^{32} (DFP^{32}) for the in vivo Determination of Red Cell Life

Span and Plasma Protein Turnover in Normal Man

By *Joseph R. Bove and Franklin G. Ebaugh, Jr.* Hitchcock Clinic, Hitchcock Foundation, and Dartmouth Medical School, Hanover, New Hampshire. (Aided by a grant from the American Cancer Society.)

Cohn and Warringa described the use of DFP^{32} as a labeling agent for measuring the RBC life span and plasma protein turnover rates in human patients. This observation, in addition to work on RBC cholinesterase regeneration after exposure to DFP, suggests that DFP may be an ideal labeling agent for measuring RBC survival. In this study 8 normal volunteers received 0.9 mg. of DFP^{32} in one gram of peanut oil I.M. No adverse reactions were noted. When exposed to air, DFP^{32} volatilizes from the peanut oil in an exponential manner with a half time of 15 minutes. The P^{32} washed and dried RBC from 2 ml. of whole blood was counted with a 0.95 mg./cm.^{2} "Mylar" window gas flow counter with an efficiency of 40% and a background of 30 CPM. 26 ± 2% (23–28) of the injected dose was present in the red cell mass and 24 ± 6% (19–33) in the plasma on day 1 after injection. The RBC P^{32} activity decreased linearly with a rate of 0.83 ± 0.08% per day or the equivalent of 120 ± 12 day mean RBC life span. The plasma P^{32} decreased exponentially with a mean rate constant of 0.042 ± .008 (.032–.053) days or a range in the half-life from 14–22 days. One mg. of DFP^{32} of specific activity 100 μc./mg. given to a 70 Kg. man with a normal RBC survival will result in enough radioactivity to give a gross counting rate of twice background 90 days after injection with the counting conditions of this study. No evidence was found for elution of DFP^{32} from the circulating erythrocytes, a distinct advantage over $Na_2Cr^{51}O_4$ as a labeling agent for measuring RBC life span in vivo.

Hereditary Spherocytosis Without Demonstrable Hematologic Abnormality in Parents

By *Russell Weisman, Jr., Carl F. Hinz, Jr. and Wayne H. Borges.* Departments of Medicine and Pediatrics, Western Reserve University School of Medicine, Cleveland.

Although hereditary spherocytosis is believed to be inherited as a Mendelian dominant, Race, Dacie, Young, and Newton independently have reported a disease indistinguishable clinically from hereditary spherocytosis which occurs in children of parents without hematologic abnormality. In this study, observation of 22 patients with hereditary spherocytosis from 18 families has revealed 7 families in which one child has clinical hereditary spherocytosis, although no hematologic abnormality has been detected in either parent. In one of the 7 families a maternal aunt has the disease but the mother of the affected child is normal.

Studies of patients and family members included complete blood indices, reticulocyte counts, serum bilirubin, and osmotic and mechanical fragility determinations on both fresh blood and blood incubated at 37° C. for 24 hours. Blood grouping was done to exclude impaternity. In 4 families erythrocyte autosurvival studies with Cr^{51} were done in parents of affected children. Normal red cell survival was demonstrated in the 7 parents studied, one of whom is the woman with both an affected daughter and sister.

The hematologic normality of parents of affected children has been explained by gene mutation in the child or by limitation of gene penetrance so that a parent is a clinically unaffected "carrier." The family in which the mother is normal, while her sister and daughter have hereditary spherocytosis, supports the concept of lack of penetrance. In the 6 remaining families with normal parents and affected offspring either mutation or lack of penetrance could be invoked to explain the disease. Evidence for lack of penetrance was not found. Mutation in each of 6 children cannot be excluded. A third possibility exists: clinical hereditary spherocytosis may occur as the result of two non-allelic genes rather than as a Mendelian dominant, although evidence to support this concept is not available.

Serum Co^{60} Vitamin B_{12} Binding Capacity in Some Hematologic Disorders

By *Robert W. Bertcher, Leo M. Meyer and Eugene P. Cronkite.* South Nassau Communities Hospital, Oceanside, and the Brookhaven National Laboratory, Upton, New York.

It has been recently reported that the range of Co^{60} vitamin B_{12} binding capacity of normal human serum may be estimated by incubating the radioactive vitamin with serum and dialyzing against running tap water for 48 hours. This binding capacity has been studied in various hematologic disorders with the following results:

Ten of 13 cases of chronic myelocytic leukemia showed an elevated binding capacity which was roughly but not regularly proportional to total leukocyte count. Those cases showing normal binding were under treatment and in remission. One of six cases of chronic lymphocytic leukemia showed moderate, and two slight increase of binding capacity. This varied with leukocyte count, but was less elevated than in chronic myelocytic leukemia. Increased binding capacity was also found in one of seven adult cases of acute leukemia, two cases of chronic, idiopathic leukopenia, one of two cases of polycythemia vera, and none of three cases of pernicious anemia in relapse.

The findings in chronic myelocytic leukemia and polycythemia vera parallel reports of increased serum content of vitamin B_{12} in these diseases.

In none of the conditions studied was binding capacity reduced.

Transfer of Iron and Cobalt from Serum Iron-Binding Protein to Human Reticulocytes

By *James H. Jandl, John K. Inman, and Richard L. Simmons.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston. (Aided by a grant from the Helen Hay Whitney Foundation.)

Since the initial step in iron utilization by immature red cells involves the transfer of iron from an extremely stable complex with the serum iron-binding protein (IBP), this interaction was investigated with Fe^{59}, Co^{60}, purified human IBP, and human reticulocytes. Whereas free Fe^{+++} readily combined with both mature red cells and reticulocytes, iron bound to IBP was taken up only by reticulocytes. Reticulocytes from other mammalian species also utilized iron from human IBP. Iron uptake was maximal when the iron saturation of IBP exceeded 25% and at lesser saturations was competitively inhibited by IBP. Iron transfer was also inhibited by HCO_3^-, most metabolic poisons, and particularly Pb^{++}. Iron uptake by reticulocytes was little affected by physiologic differences in temperature, pH, or pO_2; however, the proportion of reticulocyte Fe^{59} incorporated into hemoglobin was greatest at low oxygen tensions. The uptake of free Fe^{+++} by reticulocytes was readily prevented or reversed by EDTA, whereas even excess EDTA failed to prevent or reverse the uptake of iron from IBP. Free Fe^{+++} was bound by the membranes of hemolyzed as well as intact reticulocytes and was incorporated into hemoglobin. However, iron transfer from IBP to the reticulocyte membrane and its incorporation into hemoglobin occurred only with intact reticulocytes.

Trivalent cobalt and manganese also formed colored complexes with IBP. Unlike free Fe^{+++}, Co^{+++} was taken up more readily by immature than by mature red cells. A more selective uptake of cobalt by reticulocytes occurred when the cobalt was combined with IBP, although this uptake was consistently less than that of iron.

These studies indicate that a specific and direct interaction occurs between IBP and the membranes of immature red cells, permitting the transfer of iron and certain other trivalent metals. This mechanism is distinct from that of heme synthesis and requires cellular integrity.

Studies on the Survival of Incompatible Red Cells in Patients with Acquired Agammaglobulinemia

By *Hugh Chaplin, Jr.* Division of Hematology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.

Absence of anti-A and anti-B isoagglutinins has been considered a characteristic feature of the syndrome of agammaglobulinemia and has led to the suggestion that patients exhibiting this syndrome could be considered "universal recipients" for purposes of blood transfusion. The present study was undertaken to explore these concepts.

Four adult patients are described who were diagnosed as having acquired agammaglobulinemia on the basis of paper electrophoretic studies. All four patients could have been considered "universal recipients" because of the absence of ABO isoagglutinins on routine laboratory tests. Weak isoagglutinins were detectable in the sera from three of the four subjects when the sensitivity of the in vitro test was increased. In vivo survival of Cr^{51}-tagged incompatible red cells was shortened in all of the patients, with a wide range of halftime survivals from as short as 10 minutes to as long as nine days. The accumulation of radioactivity over the hepatic and splenic areas was measured by surface scintillation counting. Although the in vitro tests for antibody activity revealed no qualitative differences among the sera from the three patients with detectable isoagglutinins, two different mechanisms of red cell removal were observed, one of which (in two patients) entailed nearly equal participation of both liver and spleen, the other (in one patient) being almost exclusively a function of the spleen.

The extreme sensitivity of the in vivo studies as a test for minute amounts of circulating antibody is emphasized. The application of similar studies promises to aid in more precise characterization of the congenital and acquired forms of agammaglobulinemia and to expand the understanding of basic mechanisms for the destruction of ABO-incompatible cells.

The Distribution and Turnover of Parenterally Injected Co^{60}-Labeled Vitamin B_{12}

By *Donald A. Willigan, Leo M. Meyer and Eugene P. Cronkite.* Medical Department, Brookhaven National Laboratory, Long Island, and South Nassau Communities Hospital, New York; School of Veterinary Medicine, University of Minnesota, St. Paul. (Aided by a grant from the U. S. Atomic Energy Commission.)

Population studies with $Cobalt^{60}$ labeled vitamin B_{12} are being undertaken by various groups to study inherited characteristics in PA families. Furthermore, plasma clearance studies following parenteral injections are being pursued in patients. Little

is known about the somatic and genetic radiation hazard of the radiovitamin. The turnover in the liver is slow. Dogs injected intravenously via (a) the cephalic vein and (b) the portal system showed no essential difference in the uptake of radioactivity within the liver (in vivo scanning). Radioactivity excreted was highest at one to two days (urine) and three to five days (feces) after injection. Subsequently, the activity dropped precipitously (urine) and more gradually (feces) to a plateau of low excretion. In dogs with biliary fistulae, 3.59, 4.28 and 6.62% of the injected dose was excreted in the bile. The highest concentration was observed 4 to 8 hours after injection, and after 100 hours there was a low constant excretion just above background. Tissues (approximately 105 in number) from each dog were collected at necropsy. The following are listed according to highest content of Co^{60}: pituitary, adrenals, gastric mucosa (fundus), kidney, pancreas, prostate, liver, spleen, heart, testes, lung, thyroid, submaxillary gland, cerebrum, bone marrow, skin, skeletal muscle. The level in the liver (external counting) at 172–187 days decreased to 20.25 to 33.38% of the maximum radioactivity. Rats injected intraperitoneally with Co^{60} vitamin B_{12} were killed at weekly intervals for a period of 70 days. Distribution and turnover as a function of time will be presented. Assuming no biologic turnover, the 5 year beta dose to the pituitary is 70 rads and to the testes 0.95 rads.

Tissue Culture of Bone Marrow

By *Edward H. Reisner, Jr.* St. Luke's Hospital, New York.

Bone marrow grows readily in vitro but with the methods in use, the different blood cells are rapidly replaced by spindle-shaped cells called fibroblasts. These proliferate rapidly and by subculturing every few days, can be kept alive for years. Apparently, in vitro conditions favorable for growth do not permit differentiation and vice versa. It appears self-evident that a cell cannot both divide and mature. Examination of normal bone marrow smears and sections reveals a small amount of mitotic activity (growth) in proportion to the total number of cells.

We have developed a technic of marrow culture adapted from the method of Sano and Smith, that permits the maintenance of marrow as marrow for periods as long as 14 weeks. Marrow particles squeezed from freshly resected human ribs are placed at the bottom of a well formed by sealing a cylindrical glass ring (9 x 15 mm.) on an ordinary 22 mm. glass cover slip. A piece of tantalum gauze mesh cut to fit the inside diameter of the cylinder is placed on top of the marrow particle to keep it from floating to the top of the medium, which is then added to within 1 mm. of the top of the well. The well is sealed with a second cover slip. The preparation can be inverted and studied at will under the microscope. By agitation, cells from about the explant can be dispersed in the medium and removed for examination in stained smears as often as desired. The marrow explant can be readily obtained for examination in a fixed preparation by removal of the bottom cover slip.

We have experimented with various media including human serum alone, and with varying concentrations of Hanks Balanced Salt Solution, or mixture 199, and embryo extract. At present we favor 60% malignant pleural exudate and 40% mixture 199, with a drop of penicillin. The cultures are fed once a week and left otherwise undisturbed except for occasional examination. In these preparations there is virtually no fibroblast formation. Readily identifiable erythroblasts, myelocytes and leukocytes, and platelet producing megakaryocytes are released from the explant for 5–6 weeks; after this time neutrophilic elements and monocytes predominate. At 3 months the marrow explant is still readily identifiable as marrow. Most of the cells appear to be dead but there are enough well staining cells remaining to account for the continued production of leukocytes and phagocytes that exhibit metabolic activity, as evidenced by changes in pH indicator in the medium, active ameboid motion and ability to phagocytose vital stains.

It appears possible that the continued differentiation of marrow under these conditions is due to the fact that the conditions of the culture, e.g. low oxygen tension, high cell inoculum, infrequent change of medium, are not favorable enough to permit growth at any but a minimal rate, with the result that most of the cells differentiate rather than divide. An alternative explanation is that in these cultures we have fortuitously hit upon the "gradient factor" (Osgood) optimal for marrow differentiation.

In Vitro Observations on Oxygen Consumption, Heme Synthesis and Desoxyribonucleic Acid Synthesis by Pernicious Anemia Bone Marrow

By *E. Donnall Thomas and Harry L. Lochte, Jr.* Mary Imogene Bassett Hospital, Cooperstown, New York. (Aided by a grant from the U. S. Public Health Service.)

The nature of the biochemical defect in pernicious anemia (P.A.) is still not understood. Direct studies on P.A. bone marrow cells have been confined to observations of morphologic changes and cell counts in tissue cultures. In attempting to avoid the difficulties inherent in such investigations, we have studied P.A. bone marrow by the following technics: (1) Oxygen consumption, (2) Heme synthesis, as measured by the rate of incorporation of C^{14}-glycine into heme, and (3) Desoxyribonucleic acid (DNA) synthesis by measurement of the rate of incorporation of C^{14}-formate into thymine. These technics permit measurement of biochemical activity over short

periods of time and with small amounts of bone marrow.

Utilizing these methods, it was found that P.A. bone marrow synthesizes DNA at a greater rate in normal serum than in P.A. serum. Addition of vitamin B_{12} to P.A. bone marrow in P.A. serum accelerates the rate of DNA synthesis. B_{12} and folic acid have no effect on oxygen consumption or heme synthesis. Folic acid and citrovorum factor accelerate markedly DNA synthesis in some marrow samples and show no effect in others. P.A. serum has no demonstrable inhibitory effect on normal bone marrow. From these studies it is concluded that vitamin B_{12} and folic acid are specifically concerned with DNA synthesis, that B_{12} has a direct action on the marrow cells and that folic acid affects some P.A. marrows but not others.

These studies provide evidence of the importance of B_{12} in DNA synthesis by human cells. The technic described make it possible to apply the data from numerous microbiologic studies to human cells.

Immunologic Differences Between Iso- and Auto-Antibodies

By *Bernard Pirofsky*. University of Oregon Medical School, Division of Experimental Medicine, Portland.

Incomplete iso-antibodies can be demonstrated by their erythrocyte coating effect using anti-human globulin (Coombs) serum. Erythrocyte coating material characteristic of "symptomatic" autoimmune hemolytic anemia can similarly be demonstrated. Accordingly, this erythrocyte coating material has been considered an incomplete autoantibody.

The immunologic relationship between anti-human globulin serum, iso-antibodies, and the presumed auto-antibodies was studied. Anti-human globulin serum was obtained from four different sources. Anti-C and Anti-D were prepared free from serum proteins by coating appropriate erythrocytes, washing the erythrocytes, and eluting the iso-antibodies. Erythrocyte coating material free from serum proteins was obtained by the elution of washed, Coombs positive erythrocytes obtained from four cases of chronic lymphocytic leukemia complicated by hemolytic anemia. Mixtures were made containing equal volumes of anti-human globulin serum and the two types of antibodies. After refrigeration at 4° C. for 24 hours, the availability of uncombined antibody in these mixtures was determined by indirect Coombs testing and with the use of enzyme treated erythrocytes. The availability of uncombined antihuman globulin serum in these mixtures was determined by the agglutination of anti-D coated erythrocytes.

It was found that Anti-C and Anti-D reacted with the various anti-human globulin serums leading to inactivation of the isoantibodies and the anti-human globulin serum. On the other hand, when erythrocyte coating material eluted from leukemia cases was premixed with anti-human globulin serum, no loss of potency in its coating effect was noted. Similarly, there was no loss of potency of the anti-human globulin serum. When erythrocytes were added to these mixtures a marked decrease in potency of both the erythrocyte coating material and the anti-human globulin serum was noted. In spite of the apparent lack of immunologic reaction between erythrocyte coating material and anti-human globulin serum without erythrocytes, it was determined by electrophoretic fractionation that the active material is a slow-moving gamma globulin.

Investigation of The Genetic Basis for Pernicious Anemia

By *Patricia A. McIntyre*. Department of Medicine, The Johns Hopkins University, Baltimore.

Familial occurrence of pernicious anemia is so frequent as to suggest that heredity plays an important role in the development of this disorder. A group of over 100 relatives of patients with known pernicious anemia were investigated, utilizing the technic of Schilling for measuring absorption of $Cobalt^{60}$-Vitamin B_{12}. Compared with a normal group, a significant percentage of the family members had impaired absorption of Vitamin B_{12}. The data suggest that this absorptive defect is governed either by a Mendelian dominant gene of relatively infrequent occurrence in the population or by a commonly occurring Mendelian recessive gene. Analysis of individual family groups, however, suggests that the former possibility is the more likely. The wide variation of test results below the normal range suggests that this gene is of variable penetrance.

The majority of these family members were also examined for anemia, gastric acidity, blood types and sub-groups. Unlike previously reported studies, there was no significant incidence of hypochromic anemia in these relatives. Utilizing a tubeless method of gastric analysis and re-examining subjects with apparent achlorhydria with gastric intubation and histamine stimulation, we found approximately 15% of these relatives to have achlorhydria. Blood groupings showed no obvious linkage of this gene with any of four blood group systems.

Electrophoresis of Acquired Hemolytic Anemia Serum: Abnormal $Gamma_1$ Peak Composed of Cold Antibody Protein

By *W. N. Christenson and J. V. Dacie*. New York.

In a survey of the paper electrophoretic patterns of 85 sera from patients with acquired hemolytic anemia, it was found that sera derived from 9 patients with the syndrome of chronic hemolytic anemia, hemoglobinuria, Raynaud's phenomena and cold hemagglutinins in a very high titer (> 8,000 at

4° C.) produced an abnormal peak in the gamma$_1$ position. Three of these sera contained cryoglobulin (1–5% by volume), precipitable at 4° C., and migrating electrophoretically in the gamma$_1$ peak. Stimulated by the evidence that many antibodies migrate electrophoretically in the gamma$_1$ position, we undertook studies to show that the abnormal peak in these sera was produced by the high concentration of antibody.

Three sera were fractionated into samples containing a representative range of alpha$_2$, beta, gamma$_1$ and gamma globulins by means of zone electrophoresis in a Porath column. Titration of antibody activity of the samples demonstrated an overwhelming concentration of antibody in the gamma$_1$ fractions. Antibody was absorbed from two sera by serial exposure to normal group O erythrocytes in the cold. Paper electrophoresis of the concentrated supernatent from each absorption showed a progressive reduction in the height of the gamma$_1$ peak, proportional to the reduction of the antibody titer. Electrophoresis of the antibody, eluted from sensitized normal erythrocytes, demonstrated a homogenous band migrating in the gamma$_1$ position. Ultracentrifugal analyses (carried out by P. A. Charlwood of the National Institute for Medical Research) appear to confirm the observations of other workers indicating that this type of cold antibody is macromolecular. The antibody in one of the three sera studied, but not the others, also had the properties of a cryoglobulin.

These studies indicate that the high titer cold hemagglutination syndrome represents a form of paraproteinemia in which an abnormal globulin with the properties of a cold autoantibody is detectable by electrophoresis or ultracentrifugation.

Mixed Agglutination of Leukocytes and Erythrocytes in Relation to Studies of Leukocyte Antigens

By *Richard F. Bakemeier and Scott N. Swisher.* Department of Medicine, The University of Rochester School of Medicine and Dentistry, Rochester, New York.

Much investigation has recently been directed toward the serologic behavior of leukocytes, especially regarding the presence of erythrocyte antigens on leukocytes, and the demonstration of pathologic leukoagglutinins. The investigations to be reported demonstrated serious potential sources of error in leukocyte agglutination technics.

Normal leukocytes can adhere non-specifically in vitro to erythrocytes of different antigenic structure when the erythrocytes are sensitized with complement-fixing isoantibodies. If the red cells in such a system are hemolyzed by the action of antibody and complement or osmotically, and the leukocytes remain attached to the ghosts, the mixed composition of these clumps, which appear to be pure agglutinates of leukocytes under ordinary microscopy, will not be recognized. When such clumps are examined under phase microscopy, their mixed nature is revealed.

Leukocytes from donors of blood group O, as well as group A, clumped in the presence of immune human anti-A if group A erythrocytes or ghosts were present. Comparable results were found with immune human anti-B, anti-Lewis, and with a canine isoantibody of immune type, in the presence of the corresponding erythrocytes. Negative results were found with non-complement-fixing antibodies such as anti-Rh, certain human anti-A, and certain saline-active canine isoantibodies, and with all sera, except high titer immune human anti-A, when fresh serum was not present in the system. Thus it appeared that the clumping of leukocytes did not depend on the blood group of the leukocyte donor, but instead depended on the presence of a potentially hemolytic antibody which reacted with erythrocytes present in the leukocyte suspensions.

These observations indicate that clumping of leukocytes, both apparently alone and mixed with erythrocytes, must be interpreted with extreme caution when erythrocytes and erythrocyte isoantibodies are present; and that certain recent investigations of leukocyte antigenic structure, employing direct agglutination technics, should be re-evaluated.

Demonstration of a Normal Human Serum Inhibitor of Formate Incorporation into Leukocyte Protein

By *J. H. Frenster, W. R. Best and R. J. Winzler.* Departments of Medicine and Biochemistry, University of Illinois College of Medicine, Chicago. (Aided by grant from the U. S. Public Health Service.)

Human leukocyte physiology encompasses studies of (1) proliferation and differentiation, (2) release, storage, and destruction, and (3) discrete cellular function. Quantitation of the rate of leukocyte protein incorporation and investigation of its normal controlling mechanisms are important phases of such studies.

The rate of leukocyte protein incorporation in vitro was determined from specific activity of the gross protein fraction of isolated human leukemic leukocytes after incubation with formate-C^{14} in Ringer-Bicarbonate-Glucose medium. Fresh fasting normal human serum added to the medium consistently depressed formate-C^{14} incorporation into the gross protein fraction. Possible glucose, ionic, pH, protein, corticoid, and formate artefacts were excluded. Serum inhibitor is dialyzable, ultra-filtrable, heat stable, ether-insoluble, and nonadsorbable by ion exchange. Comparative interrupted incubations of leukocytes suggested that deoxycorticosterone increased cellular protein degradation and that serum inhibitor decreased cellular protein synthesis. Formate incorporation into the acid-soluble pre-

cursor pool was inhibited to a lesser degree than into the gross protein fraction.

Cells from different patients with leukemias of the same type and degree were variably responsive to the inhibitor. Analysis of variance of an experiment using 7 patients' sera and 2 patients' cell types showed significant intersera, inter-cell type, and interaction differences. Sera of 11 patients with leukemias, other leukocyte abnormalities, and dysproteinemias were tested to examine clinical variation of inhibitor levels. The greatest amount of inhibitor was present in a hypersplenic pancytopenia and a septicemic leukocytosis, while the least was found in a chronic lymphocytic leukemia and a hypoplastic pancytopenia. Such variations need further quantitation before interpretation.

These studies demonstrate that a serum inhibitor of formate incorporation into leukocyte protein is present normally and in various diseases. The significance of this normal inhibitor in physiology, diagnosis, and therapy is under study.

Leukocytic Enzymes Depolymerizing Desoxyribonucleic Acid and Ribonucleic Acid: A Preliminary Report

By *B. E. Maney, W. C. Moloney and F. H. L. Taylor.* Hematology Laboratory, First and Third Medical Services (Tufts), Boston City Hospital. (Aided by a grant from the Atomic Energy Commission.)

In the course of investigation of metabolic and enzymatic activities of leukocytes, enzymes capable of depolymerizing desoxyribonucleic acid and ribonucleic acid were separated from the leukocytes of patients with myeloid metaplasia and chronic myelogenous leukemia.

These preparations were made either by the classical procedures of Chargaff and Zamenhof, Avery and McCarty, Kunitz and MacDonald, or by new methods of isolation, such as critical concentrations of alcohol of low ionic strength in the cold and dialysis. It is obviously important to demonstrate the presence of such enzyme systems and their inhibitors, in order to establish the fact that the leukocytes exhibit a complete control of their enzyme systems. During the preparation of these fractions, control of temperature, pH, activators and inhibitors was maintained.

The activity of these enzyme systems was semiquantitatively evaluated by viscosimetric technics, using temperatures of 37° C., pH 7.0–7.4, and low concentrations of magnesium ions for the demonstration of desoxyribonucleic acid depolymerase. On the other hand, ribonucleic acid depolymerase was demonstrated at temperatures of 65° C., pH of 7.7, and in the presence of zinc ion activator. In every instance, the observation of any activity in the products obtained by the new methods was confirmed by the application of the corresponding classical technic.

These preliminary findings indicate that the leukocytes studied contained enzymes which specifically depolymerize desoxyribonucleic acid and ribonucleic acid, and support Chargaff's concept of a self-controlling depolymerizing system in human cells. Observations on the presence of a specific inhibitor, previously described by Henstell et al., are in progress.

DNases and Inhibitors in Aging and Following X-Radiation

By *N. B. Kurnick, Barbara W. Massey and Kay B. Thomas.* (Aided by grants from the American Cancer Society, U.S.P.H.S., and Los Angeles County Heart Association.)

Cell death with age and post-irradiation autolysis may be due to increase in desoxyribonuclease (DNase) activity (probably as a result of destruction of inhibitor).

DNase activity and inhibitor were determined in mouse tissues at various pH's. Spleen, kidney, and liver have maximum DNase activities at pH 5. Spleen and kidney DNase activities (pH 5) increase markedly with age, whereas liver shows no significant change. The inhibitory activity of spleen against rabbit serum DNase (pH 7.5) also increases with age, while liver activity falls and kidney shows no significant change.

Lethal x-radiation causes sharp increase in spleen acid DNase activity, reaching a peak of about 300% on the third day. This level is maintained to death. Simultaneously the inhibitor of rabbit serum DNase falls to approximately 25% of the pre-irradiation level. In animals protected by bone marrow injection 24 hours after irradiation, the acid DNase activity of the spleen reaches the same peak as in the controls, but promptly returns to normal by the seventh day. Similarly, the alkaline DNase inhibitor falls and then returns to normal. Liver and kidney activities do not change.

It is tempting to equate the post-irradiation responses of alkaline DNase inhibitor with those of an acid DNase inhibitor. Unfortunately, the inhibitor of alkaline (serum) DNase does not parallel reciprocally the change in acid tissue DNase with age, nor does it inhibit acid DNase. Nevertheless, the data suggest that x-radiation and senescence may cause (1) destruction (with replacement following bone marrow injection) of acid DNase inhibitor; (2) destruction of splenic cells poor in DNase preferentially to those rich in this enzyme (bone marrow injection effect would be ascribed to preferential regeneration of non-DNase containing cells); or (3) activation of DNase. The third is improbable, and the absence of striking histologic change during aging favors the first hypothesis over the second.

Pathogenetic Mechanisms of Congenital Neutropenia

By *Mario Stefanini and Rose H. Mele.* Joseph Stanton Laboratories, Saint Elizabeth's Hospital; Tufts University Medical School, Boston.

Repeated infections and extreme neutropenia occurred in a 22-year-old female. Bone marrow showed myeloid hyperplasia and maturation arrest. Patient's serum injected into healthy individuals induced significant neutropenia. Later, patient became pregnant; child was born with extreme neutropenia, lasting 17 days. Patient had never been transfused; yet, her serum agglutinated (a) 67% samples of normal human leukocytes; (b) child's leukocytes; (c) patient's own leukocytes. Child's serum, taken immediately after birth, agglutinated samples of normal leukocytes, mother's leukocytes and autoleukocytes. Splenectomy was performed later in the mother with slight hematologic remission; leukocyte agglutinin persisted in serum. Congenital leukopenia appeared due to transplacental transfer of leukocyte agglutinin, possibly an antibody.

A 29-year-old patient was first seen during seventh month of pregnancy. There was extreme neutropenia; bone marrow showed myeloid hyperplasia, maturation arrest. No neutrophil agglutinins or precipitins were detected in serum. No anti-neutrophil antibodies could be detected by finer technics (complement fixation, AHG fixation test, etc.); administration of patient's serum to healthy individuals did not induce neutropenia. A child was born showing severe neutropenia lasting 10 days. Bone marrow showed findings similar to those in the mother's preparation. No anti-neutrophil antibodies were detected in the child's serum. Patient was later splenectomized with poor hematologic response. Studies were then conducted to recognize an anti-neutrophil agent in various extracts of splenic tissue; these were uniformly negative. Congenital leukopenia was likely due to transplacental transfer of an unknown factor, probably too labile for extraction and detection.

Congenital neutropenia followed transplacental transfer of anti-neutrophilic factors from neutropenic mother. In one case, the agent could be fairly well characterized, fitting the attributes of auto- and iso-antineutrophil antibody.

Morphologic and Histochemical Investigation of the Leukocyte Anomaly of Chediak

By *W. W. Thayer.* Hematology Laboratory, First and Third Medical Services (Tufts), Boston City Hospital, Boston.

In 1952 Chediak described a Cuban family of 13 children, the product of a consanguinous marriage, four of whom presented albinism, anomalous giant granulation of the leukocytes and, later, leukopoietic and thrombopoietic failure, with death due to uncontrollable infection. Recently we encountered such a case, which presented an unusual opportunity to investigate the morphologic and histochemical nature of leukocyte granules.

Chediak has described the complete syndrome, with nuclear as well as cytoplasmic abnormalities present in every series of leukocytes. The present case appears to be a partial form of this anomaly, showing giant, polymorphous, granulocytic granules and variable sized lymphocytic granules. However, the monocytic and plasmacytic series appear normal, as do the nuclei of all series of white cells.

Morphology was studied, using the routine Giemsa, Wright-Giemsa, and May-Grunwald-Giemsa stains on air-dried films of peripheral blood and of bone marrow. These observations were confirmed by the study of unstained and of supravitally stained capillary films of living cells examined under bright-field and phase-contrast conditions. Histochemical studies are in progress; DNA has been studied by means of the Feulgen and methyl-green reactions, and RNA by means of the Unna-Pappenheim stain and Korson's nucleic-acid trichome. Lipid distribution has been investigated utilizing sudan IV, sudan black-B, and further studies are planned using Baker's calcium-formol, acid-hematin method for phospholids. A modification of Hotchkiss' P.A.S. reaction was used to identify carbohydrates and highly unsaturated lipids. Mucoproteins are being investigated by means of their metachromatic reaction with toluidine blue. The activity of various enzymes, including peroxidase, the Nadi reaction, alkaline phosphatase, and several esterases, is under investigation.

Atypical Myeloblasts in Acute Leukemia

By *W. J. Mitus, L. J. Bergna, I. B. Mednicoff and W. Dameshek.* New England Center Hospital, Boston.

Peripheral blood of 36 cases of acute granulocytic leukemia was studied by cytochemical methods, phase microscopy, and supravital staining for the purpose of differentiating the so-called atypical myeloblasts and promyelocytes (myelo-monocytes, paramyeloblasts, "monocytes") from so-called classical myeloblasts and promyelocytes.

The following cytochemical methods were of value in this study: 1) acid phosphatase (Gomori); 2) glycogen (Hotchkiss); 3) phosphorylase (Takeuchi). In addition, the lack of motility of the atypical myeloblasts and promyelocytes as observed under the phase microscope and in supravital preparations allowed their differentiation from monocytes.

Results: Typical myeloblasts and promyelocytes usually showed no acid phosphatase activity (0–1), while the atypical forms contained a large number of "acid phosphatase granules," sometimes obscuring

the entire cell (2+ to 4+). Glycogen and phosphorylase, the latter an enzyme associated with the metabolism of glycogen, were not present or were present in small quantities only in typical forms, whereas glycogen was present in large amounts and phosphorylase usually present in atypical forms.

From a cytochemical standpoint, the cytoplasm showed increased maturity, and as the nuclei in both typical and atypical forms are primitive (fine chromatin, nucleoli), it is concluded that asynchronism of the nuclear and cytoplasmic development exists in atypical forms.

Follow-up studies indicate that the patients with typical myeloblastic forms predominating in the peripheral blood are most likely to show a striking decrease of circulating white blood cells; with massive meticorten therapy, complete remissions are relatively common in this group.

The patients with a preponderance of atypical myeloblastic cells in the peripheral blood show little or no response to corticosteroids. Treatment with 6-MP causes a rapid fall in the white blood cell count, but the bone marrow usually does not show corresponding improvement. The prognosis in this group regarding remission is poor.

Occurrence of Acute Exacerbations in Cases of Chronic Granulocytic Leukemia

By *J. Louis, W. R. Best and Louis R. Limarzi.* Department of Medicine, University of Illinois College of Medicine, Chicago. (Aided by grant from the U. S. Public Health Service.)

All investigators who have treated a number of cases of chronic granulocytic leukemia with Myleran have noted a significant percentage terminate in acute exacerbation. Wilson et al. noted six such transformations in a series of twelve cases under treatment. Some reports have suggested that Myleran might increase this occurrence. Acute exacerbation is a common terminal event with any type of therapy. The exact frequency is not generally known.

The records were reviewed from 27 cases of chronic granulocytic leukemia first seen at the university clinics in the chronic phase between 1936 and 1953 and followed closely until death under various therapeutic regimens other than Myleran. Sixteen of these died from acute exacerbation, four from complications of therapy, and seven from other natural consequences of the disease (including infections). Death from acute exacerbation occurred in from two weeks to three years after onset of therapy.

Of our original series of 21 cases treated with Myleran 11 have died. Six died from acute exacerbation, four from complications of the disease in its chronic phase, and one from unrelated disease. Three of the acute exacerbations had received other therapy prior to Myleran. Death from acute exacerbation occurred in from eight months to two years after Myleran was started.

Thus 59% of pre-Myleran deaths and 55% of Myleran deaths were due to acute exacerbation. The difference in these percentages is not statistically significant by chi-square analysis. These data indicate that acute exacerbation was the most common termination of chronic granulocytic leukemia in the pre-Myleran era. The use of Myleran has apparently not increased the occurrence of this event.

Megakaryocte Glycogen in Thrombocytopenic States

By *Harry W. Daniell.* Walter Reed Army Medical Center, Washington, D. C.

A semi-quantitative method has been developed for evaluating the histochemically demonstrable glycogen content of megakaryocytes in aspirated bone marrow. This method consists of grading each megakaryocyte from 0 to 4, and adding the grades of 50 or more such cells. Results are expressed in terms of the grade of 100 cells as the MGS (Megakaryocyte Glycogen Score). Normal values of the MGS have been shown to fall between 20 and 80, with values above this range usually associated with accelerated erythropoiesis, and values below this range usually associated with depressed erythropoiesis. No relationship between the MGS and the platelet count of peripheral blood is demonstrable.

In 30 cases of ITP (idiopathic thrombocytopenic purpura), all MGS values fell within the normal range or above it. Thrombocytopenic patients with Banti's syndrome (3 pts.), Felty's syndrome (3 pts.), acute and subacute leukemias (5 pts.), thrombotic thrombocytopenic purpura (1 pt.), and disseminated lupus erythematosus (1 pt.) had normal or elevated MGS values except in five patients with evidence of depressed erythropoiesis. One of these had an acute pneumonitis, and the other four had recently received transfusion therapy.

In one mildly thrombocytopenic patient whose platelet count did not respond following steroid therapy or splenectomy, and whose bleeding tendency was likewise unaltered by this therapy, a diagnosis of thrombasthenia seemed justified. Her megakaryocytes were practically devoid of glycogen on each of three aspirations, with MGS values of 4, 2, and 4, respectively. She exhibited no evidence of impaired erythropoiesis at any time. These data are interpreted as indicating a disease state in which demonstrably defective megakaryocytes produce defective platelets.

"Atypical Collagen Disease" with Markedly Increased Megakaryocyte Glycogen

By *Harry W. Daniell.* Walter Reed Army Medical Center, Washington, D. C.

The MGS values (Megakaryocyte Glycogen Scores) were determined on 28 bone marrow aspirates from 27 patients with "collagen diseases." Disease entities studied included rheumatoid arthritis (15 pts.), disseminated lupus erythematosus (4 pts.), transient hypersensitivity reactions (3 pts.), rheumatic heart disease (2 pts.), "atypical collagen disease" (2 pts.), and thrombotic thrombocytopenic purpura (1 pt.). As in other groups of patients studied, the MGS values were found generally to parallel the erythropoietic rate, high MGS values being associated with augmented erythropoiesis, and low MGS values being associated with depressed erythropoiesis. The aspirations on the two patients with "atypical collagen disease" did not follow this general pattern, while the other 26 aspirations were consistent in this regard.

These two patients with "atypical collagen disease" had MGS values of 172 and 174. Values over 120 have otherwise only been observed during or following intensely active erythropoiesis. Such evidence was not present in either of these cases, and bone marrow aspirates stained with Wright-Giemsa were judged to be normal. Myeloid erythroid ratios were 3–4/1. Clinically, each patient had a chronic disease during the course of which signs of myositis, arthritis, nephritis, and dermatitis varied in intensity. Fever was prominent, skin and muscle biopsies were unremarkable, and multiple L.E. preparations were negative. Both patients responded to steroid or ACTH therapy, and have now maintained remissions without this therapy for 12 and 26 months.

Of the 26 aspirations performed on other "collagen disease" patients, 18 had normal MGS values (20 to 80). Two were elevated (96 and 92), in both cases GI hemorrhage having preceded the marrow aspiration. Six aspirations had MGS values below 20, each case probably manifesting depressed erythropoiesis. These low scores were associated with pneumonia, subacute bacterial endocarditis, septicemia, multiple subcutaneous abcesses, and active disseminated lupus erythematosus, recent transfusion therapy, respectively.

Studies of Platelet Survival by in vivo Tagging with P^{32}

By *Edward Adelson, William H. Crosby and Jack J. Rheingold.* Department of Hematology, Walter Reed Army Medical Center, and Department of Medicine, George Washington University Medical School, Washington, D.C.

A method has been developed for tagging platelets with P^{32} in vivo which involves no incubation, centrifugation or washing of the platelets. The tagged platelets are transfused from a donor subject to a recipient and their survival is followed in the recipient. The method has been used with satisfactory results in both human beings and animals.

A donor animal or human is selected. In the case of human studies the ideal donor is a patient with polycythemia. However, any human who has a normal platelet count and who is on P^{32} therapy may be used. Human donors receive an intravenous injection of 3 to 5 μc. of P^{32}. Dog donors receive an intravenous injection of 1 μc. of P^{32}. One week later, 500 ml. of blood is withdrawn from the donor into a plastic bag with sequestrene as anticoagulant. Within a few minutes (never more than one-half hour) the donor blood is transfused into an appropriate recipient who has just previously had a 500 ml. phlebotomy. At appropriate intervals, 20 ml. of blood is withdrawn from the recipient by means of a two-syringe technic, sequestrene anticoagulant, and siliconized glassware. Platelets are separated, counted by phase microscopy, washed repeatedly, plated, and counted by means of a thin-window Geiger tube. Radioactivity is expressed in counts per minute per platelet. When these results are plotted against time, a curve is obtained which represents platelet survival.

The human experiments indicate that the survival of the transfused platelets is 7 to 14 days. The dog experiments show similar survival. In the first few hours after transfusion there is a transient phase during which sequestration of the transfused platelets occurs. Experiments in hypothermic dogs, using this method, have demonstrated that the thrombocytopenia that accompanies lowering of the body temperature is also due to sequestration of the platelets. They reappear when the temperature is raised.

Use of Platelet Derivatives and Platelet Substitutes in the Management of Thrombocytopenic States

By *Mario Stefanini and Sten Kistner.* Joseph Stanton Laboratories, St. Elizabeth's Hospital, Boston, and Department of Medicine, Karolinska Institute, Stockholm, Sweden.

We have administered over 711 transfusions of platelets and platelet substitutes in various primary and secondary thrombocytopenic states. Preparations used have included (a) directly transfused fresh polycythemic and normal blood; (b) platelet-rich plasma; (c) separated and concentrated platelets; (d) platelets preserved with various technics; (e) lyophilized platelets; (f) individual fractions of platelets; (g) fractions of human and bovine brain prepared to obtain phosphotidyl-ethanolamine.

Conclusions: (a) viability of platelets is in inverse proportion to the extent of the manipulations they are exposed to; (b) hemostatic effect of platelets, however, is due in large part to stable chemical constituents and thus is not directly related to platelet viability (this finding explains the relative success of lyophilized or preserved platelets in control of thrombocytopenic bleeding and justifies their clinical use); (c) partial and specific correction of

the hemostatic defect of thrombocytopenia may be obtained by the use of platelet fractions, thus supporting the concept that individual factors may be responsible for each of the multiple functions of platelets in hemostasis. The serotonin fraction of platelets, however, has no appreciable hemostatic activity; (d) brain extracts are a good source of agents with platelet-like effect. Phosphotidylethanolamine from brain is an effective coagulant with platelet-like activity.

Localization of I^{131}-Labeled Platelet Antibody in the Rat

By *Dallas V. Clatanoff and Frederick S. Bigelow.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

A partially purified platelet antibody, capable of producing prompt thrombocytopenia in rats, was isolated from rabbit anti-rat platelet serum by adsorption of the antibody on rat platelets and elution therefrom at pH 3.0. The eluted protein fraction (a gammaglobulin by electrophoretic criteria) contained approximately 40% immunologically active antibody protein. As tested in vitro, the purified protein solution contained no erythrocyte agglutinin.

After labeling with I^{131}, the platelet antibody, given intravenously to normal rats, produced thrombocytopenia within 15 minutes. Animals were killed after one hour. Control animals that received the residual labeled protein solution after removal of antibody by prior platelet adsorption exhibited no thrombocytopenia.

In both control and antibody-treated animals the liver uptake of radioactivity exceeded that in other organs. However, liver radioactivity in the antibody-treated animal was three times greater than that in the liver of the control animal. In the antibody-treated animal the liver uptake was four to five times that of the spleen or the lung. The difference in liver uptake in the two groups is thought to represent the site of greatest localization of labeled antibody. However, in the antibody-treated animal, the spleen had a slightly greater concentration of radioactivity per mg. of tissue than did the liver.

In animals made thrombocytopenic by injections of unlabeled antibody 24 hours previously, no tissue localization of radioactivity occurred subsequent to administration of labeled antibody. It is possible that this is due either to saturation of an antibody uptake mechanism or to the fact that tissue localization requires the presence of platelets and is related to platelet agglutination and removal. Under these conditions, if platelet removal is the mechanism of tissue localization of platelet antibodies, the liver is the major site of platelet removal.

Preservation of Splenic Tissue at Low Temperature

By *Harvey Rothberg and William H. Crosby.* Walter Reed Army Medical Center, Washington, D. C.

Spleens were removed from suckling RF mice, bisected lengthwise and incubated in a beaker of 30% glycerol in saline for 30 minutes. The tissue was then frozen by immersing the vessel in a dry ice-acetone mixture for 10 minutes. After thawing, each spleen was implanted in its donor, either by dropping it into the peritoneal cavity or by placing it under the skin on the animal's back.

Microscopic study of the implants removed after 15 days indicated that the frozen spleens were capable of regeneration. Sections of the intraperitoneal implants showed definite lymphoid follicles, megakaryocytes (normally found in mouse spleen) and structures resembling sinusoids. In the subcutaneous site regeneration also occurred, but it seemed to be less complete.

Studies are in progress to determine the optimum conditions for preservation of the tissue, its viability after prolonged storage at low temperatures and its ability to function as a spleen after regeneration.

Hyperkalemia in the Myeloproliferative Syndrome

By *Ralph M. Myerson and Abraham M. Frumin.* Department of Medicine, Veterans Administration Hospital; Department of Medicine, Women's Medical College of Pennsylvania, and Department of Laboratories, Albert Einstein Medical Center, Southern Division, Philadelphia.

Elevated serum potassium values of 5.4–9.1 mEq./L. were found in thrombocythemia (3), polycythemia vera (3), and myelofibrosis with myeloid metaplasia (4). Bone marrow potassium paralleled the serum. Electrocardiograms showed no evidence of hyperkalemia. Urine and sweat potassiums were normal. No significant change was found in platelet-rich or platelet-poor plasmas.

Serum potassiums were normal in erythrocytosis secondary to lung disease and uterine fibroid and in thrombocytopenias secondary to leukemia and hypersplenism (pre- and postsplenectomy). Intravenous potassium had no effect on platelet counts of normal or splenectomized individuals. Postsplenectomy thrombocytosis had unchanged serum potassiums. A patient treated with P^{32} had a fall in both serum potassium and platelet counts.

The Recognition and Management of Megakaryocytic Myelosis—a Myeloproliferative Disorder

By *William C. Levin, David C. Miesch, D. R. Celander and M. Mason Guest.* From the Departments of Internal Medicine, Physiology and Biochemistry and the Hematology Research

Laboratory, the University of Texas Medical Branch, Galveston, Texas.

The study of three cases of megakaryocytic myelosis has emphasized the importance of the prompt reduction of platelets when extreme thrombocythemia exists. Not only are spontaneous thromboses a threat to the patient's welfare, but of equal or greater importance is the threat of hemorrhage. The successful control of this abnormality has been accomplished by the administration of radioactive phosphorous.

The mechanism of the hemorrhagic symptomatology of thrombocythemia has been studied and appears to be due to the rapid and critical depletion of prothrombin and to a lesser extent of fibrinogen and accelerator globulin, by the rapid release of platelet constituents. Such intravascular coagulation reactions may proceed without the production of thrombi. Endothelial abnormalities may be necessary for accompanying thrombus formation.

The relationship of the syndrome to other myeloproliferative diseases has been considered. The possible transformation of megakaryocytic myelosis to a frank leukemia may be a greater threat to the patient's life than hemorrhage or thromboses, providing the thrombocythemia is adequately controlled by the use of ionizing radiation or radiomimetic agents. This emphasizes the necessity of recognizing and dealing with the problem of thrombocythemia in other myeloproliferative diseases, such as polycythemia rubra vera and chronic myelocytic leukemia.

Chlorambucil in the Treatment of Multiple Myeloma

By *B. J. Kennedy*. Minneapolis.

Chlorambucil (p-[di-2-chloroethyl] aminophenylbutyric acid) is an aromatic nitrogen mustard that has been effectively employed in the treatment of chronic lymphatic leukemia and some lymphomas. Its effect on five patients with multiple myeloma has been evaluated employing metabolic studies.

The patients were stabilized on a metabolic regime. Doses of 6 to 8 mg. of chlorambucil were employed. In four patients with hypercalcemia and hypercalcuria, there was a decrease of serum calcium and urine calcium excretion to normal values. In three patients with an elevated blood urea nitrogen (B.U.N.), there was a decrease. In two of these the B.U.N. became normal. There was a decrease in hyperglobulinemia in three patients, but there was no change in the electrophoretic pattern. No significant alteration in the bone marrow was noted.

Concomitant with the improvement in biochemical studies there was relief of pain and increase in well being. No improvement in the bones on roentgen examination occurred.

It would appear that chlorambucil has provoked an alteration in the growth process of multiple myeloma as judged by metabolic studies. There was a decrease in the rate of bone destruction and improvement in renal function. However, the improvement with this agent alone was insufficient to produce a clinical improvement.

It is important to be able to demonstrate minimal changes in the growth rate of malignant tumors when evaluating new chemotherapeutic agents. Metabolic studies demonstrate such changes. Such agents hence would not be discarded, and combinations of similar agents might be further evaluated.

Erythrocytic Thromboplastin Activity

By *Martin Sanders, Leo Vroman, Martin C. Rosenthal and Nina Wolf*. Department of Hematology, The Mount Sinai Hospital, New York.

Red blood cell hemolysate has been shown to have an activity similar to platelet thromboplastin factor. In addition it has the capacity to correct the prothrombin consumption of a mild hemophilic. A study was undertaken to examine the ability of such hemolysates to replace platelets in the thromboplastin generation test (T.G.T.).

Hemolysates were prepared from washed normal red cell suspensions in saline by rapid freezing and thawing in dry ice and acetone. Whereas whole erythrocyte suspensions showed insignificant thromboplastic activity, hemolysates at a concentration of 5% in normal saline proved comparable to platelets, and such platelet substitutes as inosithin, asolectin and cephalin in the T.G.T. Thromboplastic activity was found in the erythrocyte stroma and was increased with fragmentation of the ghosts by freezing and thawing. The active material was ether soluble and ethanol insoluble and is stable on storage at 4° C. for more than a month and at −30° C. for more than 3 months. Boiling for 10 minutes reduces the the thromboplastic activity of stroma but some activity persists.

The prothrombin consumption of blood obtained from patients with thrombocytopenia, thrombasthenia, mild anti-hemophilic globulin (AHG) and mild plasma thromboplastin antecedent (PTA) deficiencies was corrected by small amounts of red cell hemolysate. However, red cell hemolysate did not correct the prothrombin consumption of blood obtained from severe AHG, plasma thromboplastin component (PTA) and PTA deficiencies.

The substitution of hemolysate for platelets in the T.G.T. of normal bloods, or bloods from severe AHG or PTC deficiency gave comparable results. In mild AHG deficiency, thromboplastin generation with hemolysate was much poorer than with platelets and thus exhibited a distinct superiority over the conventional T.G.T. in uncovering mild AHG deficiency.

Ease of preparation and remarkable stability make erythrocyte hemolysate an ideal substitute for platelets in the T.G.T.

The Effect of Erythrocytes on Coagulation. I. Observations with the Thrombelastograph

By *Marilyn S. Wells, Kenneth R. Woods and Ralph L. Engle, Jr.* Department of Medicine, New York Hospital-Cornell Medical Center, New York.

The Hellige thrombelastograph recording reflects the dynamics of the clotting process and certain physical properties of the clot formed. This instrument was used to analyze the effect of erythrocytes on coagulation, particularly with regard to clot formation resulting from the fibrinogen to fibrin reaction. The coagulation of twenty normal whole blood samples was studied and the following values obtained: reaction time 5–8 minutes, coagulation time 5–8 minutes, and maximum amplitude 40–50 mm.

Normal blood was manipulated to produce elevated hematocrits without appreciably changing the concentration of other clotting factors. When such blood was allowed to clot, the reaction time was prolonged to 9–10 minutes, the coagulation time became markedly prolonged, and the maximum amplitude was greatly diminished. Although manipulated controls produced similar changes, they were never as great as those found when the hematocrit was over 67%.

In another series of experiments, a more rigidly defined system was employed. Buffered bovine fibrinogen 200–400 mg.% was converted to fibrin by the addition of thrombin and the effects of the addition of erythrocytes in this system were observed. Properly controlled ionic strength was essential. While samples without erythrocytes showed reaction times of 7–10 minutes and maximum amplitudes of 4.5–6 mm., addition of red cells to a hematocrit of 50% reduced the reaction time to 1.9–3 minutes and decreased the maximal amplitude to 1.5–2 mm.

These findings indicate that the presence of erythrocytes may alter the dynamics of the fibrinogen to fibrin reaction, and that a less rigid clot is formed. This information obtained with the thrombelastograph suggests a new approach for determining the influence of erythrocytes per se in producing the coagulation defect in primary and secondary polycythemia.

Clotting Inhibitor and A.H.G. Deficiency in Hemophilic Families

By *J. M. Lavelle, H. S. Sise and R. Becker.* Anticoagulant Laboratory, Boston City Hospital, Boston.

The possibility of hemophilias being due to excess of an inhibitory agent has been expressed by Tocantins. Numerous reports of a circulating anticoagulant in hemophilics have been made, and the "refractory state" or "plasma responsiveness" has been considered to be the result of antibodies developed to a protein which is foreign to the recipient. A method has been developed for assay of A.H.G. and demonstration of inhibitor. The test system is based on plasma rendered devoid of A.H.G. by ageing, and of platelet thromboplastin by differential adsorption on charcoal. By addition of AC-globulin, dilute cephalin, diluted adsorbed test plasma, and calcium, the clotting time of the system is critical to A.H.G. levels of the test plasma. Mixtures of two normal plasmas approach the expected values calculated on the original A.H.G. levels of each plasma. Mixtures with plasma containing the inhibitor result in a smaller yield than is expected. Survey of 10 hemophilic patients and 5 families was done. Inhibitory effect was noted in 5 of the patients. Two who had never been transfused showed inhibitor, and one who had had repeated transfusions showed no inhibitor. In general, clinically severe hemophilia was associated with the inhibitor. Three of five carriers of hemophilia showed lowered A.H.G. levels. Three of seven potential carriers were also mildly deficient. One patient demonstrated very marked inhibition, and his mother also exhibited moderate inhibitory effect. She was the only female to show this, and the finding varied with the subject with whom mixtures were made. The variation from subject to subject could be explained on the basis of widespread inheritance in the population of an inhibitor that is transmitted as a non sex-linked recessive. The random inheritance of this in A.H.G. deficient patients may account for the inhibition seen in some cases.

Intravascular Clot Lysis with Fibrinolytic Enzymes; Clinical and Experimental Studies

By *Joseph E. Sokal, Julian L. Ambrus and Clara M. Ambrus.* Divisions of Medicine and Biology, Roswell Park Memorial Institute, Buffalo, New York.

Tests of various proteolytic enzymes administered intravenously to dogs, rabbits, and monkeys with experimentally produced I^{131}-labeled arterial and venous clots demonstrated that only plasmin (fibrinolysin) was capable of lysing these clots, in dosages that did not produce serious side effects. Clots younger than three days were completely lysed by plasmin in doses of 5–30 units/Kg. Three-day-old clots were partially lysed. Five-day-old clots were unaffected. Clots sometimes reformed in vessels damaged by the experimental procedures. This could be prevented by instituting heparin anticoagulation immediately after clot lysis occurred.

Several preparations of plasmin have been tested in patients with far advanced malignant neoplastic disease. Three types of preparations were used:

I. Bovine preparations activated with chloroform; II. Crude preparations of human origin acti-

vated with streptokinase, containing an excess of streptokinase; III. Various more purified preparations of human origin, containing less streptokinase or no streptokinase.

One to five doses of various preparations have now been given to 17 patients. All preparations produced transient leukopenia and thrombocytopenia in most patients. Bovine plasmin and most of the purified human preparations caused few or no other side reactions. All preparations containing free streptokinase caused significant side reactions, including chills, fever, rise in BMR, and hypotensive episodes.

No favorable therapeutic results were obtained in patients with venous or arterial thrombosis of more than five days duration. In the only patient with early thrombophlebitis in this series, a woman treated 24 and 56 hours after estimated onset, apparent clinical resolution of the process was obtained.

Plasmin may have worthwhile clinical application in cases of fresh arterial or venous thrombosis. Work is now under way to develop a preparation which could be used in coronary thrombosis.

The Inhibition of Active Plasmin by Normal Plasma

By *Philip S. Norman*. Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore.

Plasmin is the fibrinolytic and proteolytic enzyme of blood, which, in a purified state, effectively digests fibrin clots. However, plasma contains inhibitory substances and how plasmin digests clots in their presence needs explanation. Previously reported studies indicated that plasma has two inhibitory components. The first acts almost instantaneously and is independent of temperature, whereas the second reaction progresses more slowly and is highly temperature dependent.

To study these processes, activator-free human plasmin was prepared by activating purified plasminogen with streptokinase, then precipitating the plasmin by 1M NaCl at pH 2. The precipitate was dissolved and added to dilutions of normal plasma without activating the plasminogen contained in the plasma. The residual plasmin was determined by casein hydrolysis.

The instantaneous reaction depends on both the concentration of plasma and plasmin; some plasmin remains active at any concentration of either substance. The data conform to the law of mass action and suggest a reversible reaction coming to an equilibrium. Consequently, addition of a substrate, such as fibrin, can reverse the inhibition by competing with inhibitor for the enzyme.

Temperature dependent inhibition is best studied at 25° C. since plasmin itself is unstable at higher temperatures. The rate of inhibition depends on the concentration of plasmin as well as plasma. At 37°C. the unstable plasmin is rapidly and probably irreversibly inactivated by this inhibitor. However, undigested substrate stabilizes plasmin and protects against the inhibitor and so preserves plasmin until digestion has occurred.

Alteration in Concentration of Blood Coagulation Components Following Intravenous Administration of Estrogens

By *J. Frederic Johnson*. Department of Physiology and Pharmacology, Wayne State University College of Medicine, Detroit.

Recent reports have indicated that the intravenous administration of an estrogenic complex may have a regulating effect on hemorrhage, particularly from the upper respiratory tract. Laboratory investigation of certain coagulation factors following such a procedure has shown that there is a marked rise in the prothrombin and accelerator globulin content of the plasma, accompanied coincidentally by a fall in the antithrombin content. These changes have been noted consistently in the dog and human beings. They persist for a span of time, four to five hours, which parallels the reported clinical efficacy of estrogenic medication for hemorrhage. Such alterations of the plasma factors concerned with coagulation would tend to enhance the coagulation of the blood by increasing the amount of thrombin available. This, in combination with increased amounts of AC-globulin, should prove to be of particular benefit to man, who is relatively low in AC-globulin in the plasma. The changes observed intimate therapeutic utility of interrelationships between hormones and coagulation already suggested by variations of vascular coagulation components seen in physiologic alterations of estrogen levels.

Simultaneous Measurement of Leukocyte, Platelet, and Erythrocyte Survival with Diisopropylfluorophosphate^{32} (DFP^{32})

By *M. Pollycove, G. Dal Santo and J. H. Lawrence*. Donner Laboratory and Donner Pavilion, University of California, Berkeley. (Supported in part by the Atomic Energy Commission and the Damon Runyon Memorial Fund for Cancer Rsearch.)

Current methods of estimating leukocyte or erythrocyte survival in the blood with C^{14}, N^{15}, or P^{32} are complex and lengthy, and require large blood samples. In vitro Cr^{51} labeling of erythrocytes is unsatisfactory because of variable elution. Measurement of platelet survival with P^{32} or Cr^{51} necessitates considerable manipulation, in cross transfusion or in vitro labeling.

DFP rapidly and irreversibly binds cholinesterase, and probably other cellular enzymes, without subsequent relabeling. Recently, others have shown that DFP^{32} is bound in vivo to leukocytes, platelets, and erythrocytes. These findings have suggested

that survival of these blood elements could be measured simultaneously and with relative ease.

Two to three mg. DFP^{32}, 100–175 μc./mg., is administered intravenously. Serial measurements for 100 days are made of leukocytes, platelets, and erythrocytes separated from 20 ml. samples. After initial centrifugation, platelet-rich plasma is repeatedly centrifuged until erythrocyte contamination is negligible. Packed leukocytes and erythrocytes are separted by fibrinogen sedimentation of erythrocytes. Since erythrocytes are heavily labeled, their further purification is unnecessary. Leukocytes, however, must be essentially uncontaminated. This is done by gramicidin-lysolecithin hemolysis. Leukocyte and platelet activity is measured with a modified windowless flow counter of 80% efficiency, 20 cpm background.

Studies are complete in 4 patients, continuing in 7. Leukocyte, platelet and erythrocyte radioactivity in 6 normal subjects decreased linearly, end points at 15–20, 9–11, and 105–125 days, respectively. Two patients with chronic lymphatic leukemia showed a large additional leukocyte component, end point 35 days. In a patient with polycythemia vera and another with idiopathic thrombocytopenic purpura, this second component was small. Platelet activity in 3 patients decreased exponentially with shortened survival. Disappearance of erythrocyte activity was normal, except in leukemic patients, end point 90–100 days.

These results agree with other preliminary findings and established erythrocyte data. This study indicates that DFP^{32} can be used for direct, simultaneous determination of leukocyte, platelet, and erythrocyte survival.

Erythropoietic Activity in the Plasma of Patients with Polycythemia

By *R. K. McCombs, A. N. Contopoulos, J. H. Lawrence and M. E. Simpson.* Donner Laboratory and Donner Pavilion, and Institute of Experimental Biology, University of California, Berkeley.

The erythropoietic content of plasma from patients with polycythemia vera and secondary polycythemia was compared with plasma from normal subjects and patients with the polycythemia of stress. Blood was withdrawn into heparinized, evacuated donor flasks and centrifuged, and the plasma obtained was extracted for erythropoietic activity by a method similar to that described by Borsook et al. and by Gordon et al. In many instances, several venesections were performed on the same individual, and the dialysates were pooled prior to lyophilization. The lyophilized powder was dissolved in normal saline in a concentration of 30 mg./ml., equivalent to 10–12 cc. of native plasma. Assay for the erythropoietic activity was performed in hypophysectomized rats 45 days after operation. Each rat received daily injections of the extract from the same individual for 14 days. At the end of the injection period the red cell volume of the rats was determined by the Fe^{59} method, and the hematocrit, hemoglobin, red cell count and reticulocytes were also determined.

It was found that the plasma extracted from 14 patients with polycythemia vera stimulated erythropoiesis in the recipient rats by all of the above criteria. Samples from the same individual tested at different intervals were always positive. Red cell volumes were increased by 25–30% over the control values, and the reticulocyte counts were increased from 0.3 to 8%. Extracts from 4 other polycythemia vera patients showed no definite evidence of erythropoietic content activity. Plasma from patients with secondary polycythemia also showed erythropoietic activity. Plasma from normal subjects and from patients with the polycythemia of stress elicited no increase in the hematologic values of the recipient animals. No evidence of stimulation of the adrenals, thyroids or ovaries of the injected animals was found. Source of production of this activity and identification are now under investigation.

BLOOD PROTEINS

Requirements for Optimal Resolving Power and Reproducibility in Protein Fractionation by Starch Gel Electrophoresis

By *James H. Pert, Marvin H. Sleisenger, Kenneth R. Woods and Ralph L. Engle, Jr.* Department of Medicine, The New York Hospital-Cornell Medical Center, New York. (Supported by a grant-in-aid from the National Institute of Health and the Multiple Sclerosis Society.)

Using starch gel zone electrophoresis described by Smithies, we have separated 15 to 20 protein fractions in normal sera. Adequate reproducibility with optimal protein fractionation requires rigid control at each step in the procedure. Satisfactory results depend upon the particular potato starch employed and upon the following critical limits in its preparation and use. The duration of both the acetone-HCl hydrolysis and the aqueous hydrolysis must not vary more than 5%, and the acetone temperature must be kept within 0.2° C. at 36° C. The gel buffer pH must be within ±0.01 pH units and the buffer concentration ±0.0005 molar boric acid. Significant changes will result if the percentage

of starch varies beyond 0.1%. During electrophoresis we have used 90 volts over a 20 cm. strip for 16 hours at 12° ± 1° C. Temperatures below 4° C. impede mobility, and room temperature is satisfactory only for short runs (less than 8 hours). Improved technics for staining and instrumentation for densitometry have been devised.

In addition to differences in serum haptoglobin type, there is considerable variation among normal sera. Multiple specimens from the same individual, however, prove remarkably similar. A wide variety of abnormalities in diverse disease states has been observed. Determining the specificity of the changes in a given disease and the significant differences between minor pathologic changes and normal variation will require considerably larger numbers of determinations than has been needed for less sensitive methods.

In testing for artifacts, we have analyzed a number of purified proteins by ultracentrifugal, immunologic, and electrophoretic methods. In most of these more fractions were found with the starch gel; however, the highly purified protein, ribonuclease A, prepared by C. H. W. Hirs by column chromatography, appeared as a single fraction in starch gel electrophoresis.

This method is unusually sensitive for separating the components in protein solutions. When a highly purified protein was analyzed, a single peak was observed and no artifactual fractions were detected.

Electrophoretic Demonstration of a Non-hemoglobin Protein (Methemoglobin Reductase) in Hemolysates

By *Lawrence Lonn and Arno G. Motulsky.* Department of Medicine, University of Washington, Seattle.

In studies on the heterogeneity of normal human hemoglobin, a previously unreported electrophoretic component was discovered when hemolysates (about 10% hemoglobin concentration) were subjected to paper electrophoresis at acid pH and stained with bromphenol blue. At pH 5.8 (phosphate buffer) the component was significantly slower than hemoglobin A and migrated with the mobility of hemoglobin H. On quantitation it comprised less than 1% of the total hemolysate protein. The component was present in normal amounts in patients with hemoglobinopathies and with various hematologic and other disorders. However, electrophoresis of 70 cord bloods showed the component to be greatly diminished. The fraction could be isolated from adult but not from cord blood at 72.5% ammonium sulfate. It could be established that the compound was not an electrophoretic artifact nor a stromal constituent.

The following experiments ruled out that the fraction was a hemoglobin: (a) it could not be stained with benzidine, (b) neither Cr^{51} nor Fe^{59} labeled the fraction in vitro or in vivo respectively, (c) electrophoresis of hemolysates previously exposed to various temperatures revealed disappearance of the fraction at 65° C. while the amount of hemoglobin determined colorimetrically showed no change at this temperature.

The electrophoretic mobility of the component at various pH levels was unlike that of hemoglobin A or H. However, the mobility of TPN dependent methemoglobin reductase isolated from human red cells was essentially identical to that of the component over a wide pH range. It was therefore concluded that the component represented red cell methemoglobin reductase. It is not completely excluded that other red cell enzymes have a similar electrophoretic mobility.

These studies demonstrate (1) that electrophoretic technics may be useful for the demonstration of non-hemoglobin enzyme proteins of the red cell, and (2) that rigid criteria should be satisfied before labeling small electrophoretic components of hemolysates as heterogeneous hemoglobins.

Base Binding Property of Serum Proteins for Calcium

By *Ananda S. Prasad and Edmund B. Flink.* Medical Service, Veterans Administration Hospital, Minneapolis.

A study of the base binding property of serum proteins was undertaken. Subjects were divided into four groups. Group I had normal serum calcium and proteins; Group II had multiple myeloma; Group III had hypoproteinemia and Group IV had hyperglobulinemia due to causes other than multiple myeloma. Total and ultrafiltrable calcium were determined using the technic reported by us previously. Serum protein fractions were determined by paper electrophoresis. Non-ultrafiltrable calcium in mM./Kg. H_2O was plotted against the different fractions of the proteins in percentage. Curves were drawn using the method of least squares. Albumin bound roughly 55% of the non-ultrafiltrable calcium in normals. As a rough approximation, 1 mM. of albumin bound 1.23 mM. of calcium. The general relationship between albumin and calcium remained the same in all groups, as determined by the slope of the curve. Very little calcium was bound to alpha globulin in any group.

Betaglobulin bound appreciable amounts of calcium in groups I, II and IV. In group III the binding property of betaglobulin was changed so that as the beta fraction increased less calcium was bound per unit of beta globulin. Gamma globulin bound very little calcium in group I but bound appreciable amounts in groups II and III. In group IV the binding property of gamma globulin was changed so that as this globulin increased a smaller amount of calcium was bound per unit of gamma globulin.

In the normal person roughly 5% of non-ultra-

filtrable calcium was bound to gamma globulin and nearly 40% was bound to alpha and beta globulin and probably to cephalin also, as reported by others.

In Vitro Binding of Ca^{45} to Serum Proteins Separated by Continuous Electrophoresis

By *Philip C. Johnson, William O. Smith and Charles L. Cahill.* V.A. Hospital, Oklahoma City.

That about 50% of blood calcium is bound to the serum proteins is well established. Ammonium sulfate fractionation studies have shown that both globulin and albumin are involved in this binding. By continuous paper electrophoresis (Shetlar, Cahill, Stidworthy and Shetlar), we have attempted to further clarify which particular fractions of protein possess this binding capacity.

The sera from four healthy males have been separated into 20 fractions. Ca^{45} as $CaCl_2$ was added to each fraction and the tubes were incubated for 24 hours at 37° C. Unbound calcium was removed from the fractions by 24 hour dialysis. Three major sites of calcium binding were found in the fractions representing alpha 2 globulin, beta globulin and an unnamed fraction with a faster mobility than albumin. Little binding was noted in the conventional albumin fraction and gamma globulin.

This unnamed fraction beyond albumin, although present in small concentration, showed the greatest specific activity. It gives a positive biuret and ninhydrin reaction and does not appear to contain carbohydrate.

Some Observations on Hyperglobulinemic Purpura (Waldenstrom's Syndrome)

By *William E. Symon, Robert J. Rohn and William H. Bond.* Department of Medicine, Indiana University Medical Center, Indianapolis.

Abnormal capillary bleeding and chronic recurrent, purpuric states may be associated with, and be the result of, precedent formation of various abnormal serum proteins.

The abnormal serum proteins are globulins and usually migrate with, or near, the gamma-globulin on electrophoretic migration. These abnormal globulins are roughly divided into three categories (1) macroglobulins (2) cryoglobulins and (3) hyperglobulins. All three may be associated with bleeding abnormalities.

Three cases manifesting hyperglobulinemic purpura were studied. In one, the disease was well advanced, demonstrating the classical clinical and laboratory alterations which have been outlined in the previous 18 published cases of this type. In the second, a shorter clinical course was associated with variability in clinical and laboratory manifestations, whereas the third is unique in being the first reported case of this disorder associated with and possibly due to a disseminated reticulum cell sarcoma.

Studies made included hematologic studies, hepatic function tests, clinical photographs, photomicrographs, representative free boundary electrophoretic and paper electrophoretic patterns, and ultracentrifugal patterns.

Nonmyelomatous Paraproteinemia

By *Ernest W. Smith.* Baltimore.

The serum electrophoretic pattern of 13 individuals who did not have myeloma was characterized by the presence of an abnormally well-defined globulin, or paraprotein peak. Clinical and chemical studies were made in an effort to evaluate the significance of such globulins. Some individuals were clinically well; others had diseases such as angina pectoris, tertiary syphilis, macrocytic anemia, or chronic pulmonary infections, and two had an illness which was similar to cases described as "Waldenstrom's macroglobulinemia." A slight plasmacytosis of bone marrow was encountered in most, but in those patients who were studied for four or more years no progression of plasmacytosis or of clinical illness was apparent. A very high incidence of chronic pulmonary disease, including old, extensive fibrotic tuberculosis, bronchiectasis, and, in three instances, diffuse pulmonary fibrosis, was particularly noteworthy. Measurable globulin abnormalities in addition to the abnormal electrophoretic peak included "agammaglobulinemia," cold precipitability, water precipitability and apparently large molecular size. In some patients the electrophoretic paraglobulin peak was the only demonstrable serum abnormality. In serum of others of the group, the above-mentioned abnormalities occurred singly or in various combinations. Macroglobulins of varying size were encountered among the more severe cases. From consideration of these cases, one is led to consider the possibility that nonmyelomatous paraglobulinemia might represent a response to chronic inflammation rather than a primarily malignant disorder. Characteristics of these paraproteins such as water or cold precipitability, the development of heavier than normal protein molecules, variability of electrophoretic mobility, and of polysaccharide content and absence or diminution of normal serum globulins, are conditions which are variable among individuals with similar clinical states and mitigate against rigid clinical characterizations such as "essential cryoglobulinemia" and "macroglobulinemia."

The Effect of Rabbit Plasma Protein Fractions on the Complement Fixation Reaction, Utilizing Rabbit Antibody to Sheep Cells

By *William R. Merchant, Paul Maurer and Thomas Gigliotti.* V.A. Hospital, and the Departments of Medicine and Pathology, University of Pittsburg, Pittsburgh.

A reproducible spectrophotometric complement

fixation test was selected, utilizing a standardized sensitized sheep erythrocyte suspension with optimal amounts of calcium and magnesium ions. Lyophilized guinea pig serum was used as a source of complement, utilizing five times the amount which would produce 100% hemolysis. Rabbit plasma was fractionated by Cohn's method six.

The protein fractions were added and recorded as mg./ml. of the total reacting volume. Lysis was inhibited by rabbit plasma fractions IV-4 and V proportional to their concentration. However, the relationship was not linear. Six mg./ml. of fraction V caused complete inhibition of hemolysis. The same concentration of IV-4 produced almost complete inhibition. These concentrations are within physiologic ranges. Fraction II–III did not have a great effect until a concentration of twenty mg./ml. was reached. Bovine and human albumin produced complete inhibition but at higher concentrations than observed with rabbit albumin. Rabbit plasma in high concentration produced partial inhibition.

Barbital buffer has been used as a diluent in previous methods to control pH. In these experiments it was found that in the presence of the usual barbital buffer, the addition of various protein fractions would not inhibit hemolysis. If the protein concentration relative to the barbital was increased (or concentration of barbital decreased), it was possible again to demonstrate inhibition. In this respect there was a linear relationship between the concentration of barbital and that of protein. Another urea derivative, guanidine, had a similar effect. However, if the buffer was replaced by physiologic saline (keeping pH constant at 7.2), changes in the degree of hemolysis were observed.

Heating the protein fractions to 56° C. for one hour did not affect the inhibition, suggesting that this is a heat stable factor.

These in vitro observations would suggest that the activity of complement may be modified by the various protein fractions, particularly albumin. This inhibitory action can be blocked by certain compounds, the linear relationship suggesting a chemical reaction or binding with the protein.

The Identification of the C-Reactive Protein and Various Antibodies in the Serum Gamma Globulin Obtained by Continuous Flow Paper Electrophoresis

By *R. J. Roantree, F. A. Pezold, and Lowell A. Rantz.* Departments of Medical Microbiology and Medicine, Stanford University School of Medicine, Stanford and San Francisco.

The technic of continuous flow filter paper electrophoresis was explored as a method for locating the electrophoretic fraction in which the C-reactive protein and several antibodies are found. Each electrophoretic run lasted 18 to 24 hours, using an early model of Durrum design. Constant current at about 6 ma. and a voltage of 350–450 volts were employed. Both barbitol buffer pH 8.6 and phosphate buffer pH 7.2 were used. The latter gave better antibody yield. Ionic strength varied with the run from 0.006 to 0.012. The curtain was oven-dried and stained with bromophenol blue in the standard way. If the experiment was crucial, the curtain was air-dried and stained with Oil Red O, and then counterstained with bromophenol blue. In this way the boundary between the beta-lipoprotein and gamma globulin could be demarcated. Fluid was collected from the crip points of the curtain and tested for the antibody or protein in question. Fractions collected, concentrated by dialysis and run on the standard Durrum hanging strip apparatus helped confirm the purity of the fractions.

Typhoid O, Salmonella group C, Brucella, heterophil and cold agglutinins were located in the gamma globulin. Antistreptolysin O and the C-reactive protein were also in this fraction.

The location of the C-reactive protein is the only questionable finding, since it has previously been described as an alpha and a beta globulin and has been found in two cases of agammaglobulinemia. However, it was located in the gamma fraction on 14 separate runs using sera from 8 patients with different diseases. On 3 occasions the fraction with the greatest concentrations of C-reactive protein corresponded to that with the greatest antistreptolysin concentration and, on one occassion, to that with the greatest concentration of Salmonella group C agglutinin. Hedlund has also recently found the C-reactive protein in the gamma fraction.

CARDIOVASCULAR SYSTEM

Preparation and Spectral Properties of a DPNH-succinate Cytochrome C Reductase from Heart Muscle

By *Murray Rabinowitz, Benedetto DeBernard and Ronald Estabrook.* Enzyme Institute, University of Wisconsin, Madison, and Johnson Foundation, University of Pennsylvania, Philadelphia.

The energy for cardiac muscular contraction is derived principally from high energy phosphate bonds, the synthesis of which is coupled to electron transport. The electron transfer system (ETP) of beef heart muscle has been cleaved by cholate-$(NH_4)_2SO_4$ fractionation to obtain a particulate DPNH-succinate cytochrome *c* reductase. This

cytochrome *c* reductase is equally and additively active with DPNH and succinate or substrates. It is essentially free of cytochrome *c* and of cytochrome oxidase, the principal functional components present being cytochromes *b* and c_1, and the flavoproteins associated with DPNH and with succinate dehydrogenases. Phospholipid and non-heme iron are also present. Succinate and DPNH are equally capable of reducing the cytochrome pigments present, cytochrome *b* reduced to 65% and cytochrome *c* to 80% of maximal chemical reduction. No further reduction is achieved by the addition of the second substrate to material already reduced by DPNH or succinate alone.

The less complex nature of this material as compared to ETP or to mitochondria has permitted its convenient use for kinetic spectrophotometric studies aimed at elucidating electron carrier sequence and interreaction. Using Chance's rapidly recording, dual monochrometer spectrophotometer the rates of reduction of the respiratory pigments were measured. Cytochrome c_1 was reduced 3 to 5 times as fast as cytochrome *b*, and added cytochrome *c* was reduced 3 to 5 times as fast as cytochrome c_1. Flavoprotein reduction rate was at least as rapid as that of added cytochrome *c*. These observations indicate the presence of direct flavoprotein interreaction with all three cytochromes in this preparation.

Metabolism of the Arrested and Fibrillating Perfused Heart

By *A. Beuren, J. Skibinsky, C. Sparks and R. J. Bing.* Washington University Medical Service, V.A. Hospital, St. Louis.

Myocardial extraction and usage of oxygen, glucose, pyruvate, lactate and ketones were studied in 26 arrested and fibrillating dog's hearts. In *open chest* experiments, the heart was perfused through the left coronary artery from a reservoir. Flow was determined with a rotameter. In *closed chest* experiments, both coronary arteries were perfused through a metal catheter inserted into the carotid artery. Two balloons, when inflated, directed the blood exclusively into the coronary arteries. Coronary vein blood was collected from the coronary sinus. Determination of the myocardial usage of oxygen and substrates were first carried out in the normally beating heart and after perfusion of the coronaries, during normal sinus rhythm, ventricular fibrillation and cardiac arrest. In *open chest* experiments, coronary sinus outflow, myocardial oxygen extraction and usage were significantly reduced during cardiac arrest. Fifteen minutes following cardiac arrest, myocardial oxygen usage had dropped to a tenth of its control value (0.2 cc. of oxygen/left ventricle). Values for pyruvate and lactate in coronary sinus blood exceeded those in the "arterial" blood even during the control period. Myocardial glucose balance also became negative. In *closed chest* experiments, myocardial usages of oxygen also diminished during fibrillation and arrest; however, absolute values remained higher than in the open chest experiments. (Mean of 2.5 cc. oxygen/heart). Myocardial extraction of substrate became markedly negative and glucose concentration in coronary vein blood exceeded that in arterial blood by 50 mg. %. These results illustrate the difficulties in evaluating metabolic data on the perfused heart in open chest animals; they show the marked degree of glycolysis and glycogenolysis, which operates in ventricular fibrillation and cardiac arrest.

Variability of Conventional VCG Lead Systems and ECG Limb Leads

By *Hubert V. Pipberger and Lawrence S. Lilienfield.* Cardiovascular Research Laboratory, Department of Medicine, Georgetown University Hospital, Washington, D. C.

Discrepancies between anatomic and electrical lead axes have been shown repeatedly. Schmitt and Frank have devised corrected orthogonal and weighted lead systems. These were designed from torso model studies.

Schmitt, Frank, tetrahedron and cube systems were applied consecutively to 33 normal subjects and 15 cardiac patients in order to test the performance of corrected systems in human beings and to compare them with conventional systems. Schmitt's leads, having been proved most insensitive to heart dipole changes in models, were used as reference for correlations. Frontal plane data of the tetrahedron system were used as representative for standard limb leads. Mean differences and ranges from amplitude and direction were calculated for instantaneous vectors in each third of QRS to study consecutive changes.

Amplitude data varied over a large range but minimally with Frank's system. Mean amplitudes for each lead corresponded to model data but standard deviations were 2 to 10 times higher. This indicated a marked increase in amplitude variability in humans as compared to models.

Differences in direction of instantaneous vectors were called positive when clockwise, negative when counterclockwise. In the sequence of frontal, sagittal and horizontal planes, mean differences were: $+3° \pm 15.3$, $-4° \pm 24.6$, $+5° \pm 19.2$ for Frank; $+2° \pm 31.1$ (standard limb leads), $-12° \pm 45.5$, $+9° \pm 33.1$ for tetrahedron; $+3° \pm 27.6$, $-17° \pm 41.6$, $+23° \pm 35.6$ for cube.

Mean angular differences increased and ranges decreased when data were grouped according to time and direction of instantaneous vectors. Differences between the groups were significant. This suggested that direction changes of effective lead axes occurred during QRS. Cube, tetrahedron and Frank systems were sensitive to these changes in decreasing order.

These studies demonstrate that direction

changes of effective lead axes during ventricular activation are the main cause for the large range of angular variability in conventional leads. Corrected leads significantly reduce this variability.

Upright QRS Complexes in Lead V4R in Normals

By *Gerald H. Whipple and Jay D. Coffman.* Massachusetts Memorial Hospitals and the Department of Medicine, Boston University School of Medicine, Boston.

It is well-known that some patients with right ventricular hypertrophy may show obvious electrocardiographic evidence of it only in special leads from the right chest. However, when lead V4R was taken routinely, complexes which would be interpreted as indicating right ventricular hypertrophy by existing criteria were found in a small number of normal individuals. To evaluate these observations, leads were taken from the fourth and sixth interspaces as well as in the fifth intercostal space (V4R) in the right mid-clavicular line in 540 consecutive adult patients.

QRS complexes of Rs or monophasic upward R configuration in V4R were found in 7 patients with no clinical evidence of cardiovascular or pulmonary disease, most often in females with electrically vertical hearts. Two other clinically normal patients showed rsR′ configurations in this lead, with QRS complexes of normal duration. Complexes of the various types referred to were found in normals one interspace below V4R in 15 instances, but were found one interspace higher than V4R in only 3 cases. The same decreasing incidence of dominant R waves as the electrode was moved cephalad was found in patients with probable right ventricular hypertrophy.

It is concluded that one cannot separate the QRS complexes of normals from those with right ventricular hypertrophy in an absolute fashion by the use of V4R; a distinct overlap exists. Particularly when the placement of V4R is a little low, but also when it is correct, one selectively picks up a number of cases with probable right ventricular hypertrophy as well as a few normals. Normals appear to be almost totally excluded by placing the lead one interspace higher than V4R, but such a lead is also considerably less sensitive to actual right ventricular hypertrophy.

A Comparison of Lithium and Potassium Intoxication in the Dog

By *R. Tarail, T. E. Bennett, and M. A. Bender.* Roswell Park Memorial Institute, Buffalo, New York.

Intravenous infusions of 0.15M KCl (10 dogs) or 0.30M LiCl (7 dogs) were given at 0.5 ml./ Kg./ min. during pentobarbital anesthesia. Blood samples were removed at frequent, regular intervals; continuous electrocardiograms (Lead II) were recorded. Serum potassium was measured by flame photometry in the ten animals given potassium chloride and by radioassay, after equilibration in the animals of K42 injected about 20 hours prior to infusions, in 4 of the 7 dogs which received lithium.

Animals survived 115 minutes, on the average, during lithium injection, in contrast to a mean of 45 minutes during potassium administration. Electrocardiographic changes were remarkably similar and in both groups evolved as serum potassium concentrations rose; they included disappearance of the P wave and marked auriculo-ventricular and intraventricular conduction delay. Bradycardia and QRS widening were much more pronounced after injection of potassium.

Major electrocardiographic changes began at significantly lower concentrations of serum potassium in the lithium group (mean potassium of 6.8 mM./L.) as against their onset at a mean serum potassium of 9.5 mM./L. in the potassium group. Lithium animals died with an average serum potassium of 8.0 mM./L., whereas serum potassium averaged 16.8 mM./L. at death after KCl infusions.

Therefore, although elevation of serum potassium is probably an important correlate of intoxication with lithium chloride, either lithium or the excess chloride or both are critical concomitants.

Sequence of Ventricular Contraction During Ventricular Premature Beats in Man

By *Philip Samet, Leonard Silverman, Robert Litwak, William Bernstein and H. Turkewitz.* Cardio-Pulmonary Laboratory, Mt. Sinai Hospital, Miami Beach, and the Departments of Medicine and Surgery, University of Miami School of Medicine, Coral Gables, and Jackson Memorial Hospital, Miami.

During the course of combined right and left heart catheterization, right and left ventricular pressure pulses can be recorded simultaneously. In ten such studies, 65 ventricular premature beats (with widened abnormal QRS complexes) were obtained concomitantly with bilateral ventricular pressure curves. Should mechanical asynchronism in the onset of ventricular isometric contraction accompany the electrical asynchronism of a ventricular premature systole, one ventricular upstroke would be delayed relative to the other. Left heart catheterization was performed by a modification of the posterior percutaneous puncture technic of Fisher. Right heart catheterization was performed in the usual manner.

The predominant rhythm was sinus rhythm in 5 patients; atrial fibrillation was noted in the other 5 subjects. In 9 individuals, the relationship between the onset of the "normal" right and left ventricular isometric contraction periods varied from −.02 to +.03 seconds. That is, the left ventricular upstroke

(in the nonventricular premature beats) could be .03 seconds ahead of the right; at the other extreme of the normal range, the right ventricular upstroke could be .02 seconds ahead of the left. In the tenth patient, the onset of the left ventricular curve preceded that of the right ventricle by .06 second. The site of origin of the ventricular premature beat was usually determined from simultaneously recorded leads 1 and V5 or V6.

Mechanical asynchronism, defined as differences in onset of right and left ventricular isometric contraction greater than those given above, was observed in only 28 of the 65 beats studied. In 35 instances, the difference in left and right ventricular upstrokes fell within the normal range. In 2 complexes, the onset of right ventricular contraction preceded that of the left ventricle by .03 second, despite the origin of the premature beat in the left ventricle.

These data suggest that mechanical asynchronism in ventricular contraction is not a necessary consequence of the asynchronous electrical depolarization noted in ventricular premature systoles.

The Demonstration of Flow Murmurs by Intracardiac Phonocardiography

By *Howard L. Moscovitz, Ephraim Donoso and Ira J. Gelb.* (With the technical assistance of *Walter Welkowitz.*) Department of Medicine, Mount Sinai Hospital, New York.

It has been postulated that marked acceleration or increase in magnitude of blood flow is the mechanism for the production of murmurs found in anemia, tachycardia, hyperthyroidism and pregnancy. Intracardiac phonocardiography provides direct confirmation that murmurs can be produced by abrupt changes in blood flow.

A double-lumen microphone catheter utilizing a barium titanate pickup was passed into the cardiac chambers and great vessels in a group of 45 dogs. Pressure pulses recorded through the hollow lumen of the catheter were used to precisely localize the microphone. Pressure tracings, heart sounds and electrocardiograms were recorded simultaneously on an oscillographic recorder to correlate mechanical, acoustic and electric events of the cardiac cycle.

During multiple premature ventricular contractions, flow murmurs, recorded in the great vessels, accompanied normal sinus beats following long compensatory pauses, but not frustrate beats. It appeared unlikely that the source of these murmurs was incomplete atrioventricular valve closure since the murmurs were recorded in the aorta or pulmonary artery, were produced by the sinus beats and were absent when ventricular contraction failed to open the semilunar valves.

In experimental mitral stenosis, when the microphone was positioned in the left ventricle or aorta the first ventricular contraction after release of the stenosis was accompanied by a high intensity systolic murmur. This apparently was due to the sudden augmentation of blood flow through the left ventricle into the aorta. In experimental aortic or pulmonic stenosis, systolic murmurs were detected in the great vessels during partial constriction. These murmurs diminished in intensity when blood flow was decreased by almost complete stenosis and reached their greatest intensity during the first heart beat after release of the constriction. The fact that the loudest murmurs immediately followed release of the constriction emphasized the importance of sudden increases in blood flow in the genesis of cardiac murmurs.

Cardiovascular Effects of Total Circulatory Occlusion and Release During Experimental Hypothermia: Probable "Critical" Cooling Level.

By *Emil Blair.* U. S. Army Research and Development Unit, Fitzsimons Army Hospital, Denver.

It has been observed in human beings cooled to 29–32°C. that, following release of total circulatory occlusion, an "overshoot" of the blood pressure develops, at times associated with reflex bradycardia. Those patients cooled below 28°C. failed to show these responses. The observations suggested a possible "critical" level of hypothermia at which disturbances of the myocardium and the baroceptor mechanisms are likely to develop. Studies in dogs were initiated to explore the problem under more controlled conditions. The animals were divided into the following groups: A—Control normothermia; B—"Moderate" hypothermia (35–29°C.); C—"Deep" hypothermia (28–25°C.). The occlusion was maintained for three minutes in all groups.

Occlusion: All Groups—Blood pressure (B. P.) dropped precipitously. Venous pressure (V. P.) rose gradually, reaching maximum at end of the occlusion period. Heart rate (H. R.) accelerated early and slowed during the third minute. The latter change was abolished by vagectomy or carotid degeneration.

Release: Group A—B. P. "overshoot" with reflex bradycardia appeared within 30–45 seconds. The bradycardia was abolished by vagectomy or carotid degeneration. Prostigmine did not affect the "overshoot" but masked the bradycardia. V. P. dropped promptly to normal. Group B—Phenomena same as in Group A in three of every four dogs. Group C—No "overshoot" was observed. H. R. slowed in ⅓ of the dogs, with no change following vagectomy. V. P. fell promptly.

The "overshoot" following release of total circulatory occlusion results from increased stroke volume; hence, increased myocardial work. It has been demonstrated that cardiac mechanical efficiency is markedly reduced during deep hypothermia, probably due to a lower resting myocardial

metabolism. This may account for the absence of the "overshoot" in the deeply cooled patients and animals. The bradycardia is a reflex response to the hypertension and is present during hypothermia except at deep levels. This suggests some disturbance of baroceptor mechanisms during deep hypothermia. Within the limits of this study, the experimental evidence confirms a probable "critical" level of hypothermia (28–29°C.) below which disturbances of the myocardium and the cardiovascular baroceptors are likely to occur.

Measurement of Transcoronary Circulation Time (TCT) by Radioactive Isotope Technics

By *Richard Gorlin and John P. Storaasli.* U. S. Naval Hospital, Portsmouth, Va.; Peter Bent Brigham Hospital, Boston; and University Hospitals, Cleveland.

The current methods of studying the coronary circulation in man are limited by the long period of time and the excessive removal of blood necessary for each determination. Both limitations have been obviated by a new radioisotope technic which gives circulation time through the coronary vascular system draining the left ventricle. A scintillation counter, with little collimation, is located over the anterior thorax usually to the left of the midline between the third and fifth interspaces. A standard cardiac catheter is located in the coronary sinus. 10–20 μc. of radioactive iodinated serum albumin is injected into an arm vein. Arrival over the right and left heart chambers is detected by the surface counter from which a typical two-humped radiocardiographic curve is written. The isotope is next detected from the catheter either via multiple syringe sampling or continuous sampling past a second scintillation counter. Transcoronary circulation time (TCT) is measured from arrival over the left heart to arrival at the catheter (corrected for delay in withdrawal).

In 15 normals and patients with mild heart disease, TCT varied from 6.5 to 11 seconds. Nitroglycerin, exercise, and atropine cardioacceleration decreased this time, while Valsalva's maneuver increased it. In all cases where TCT decreased, the simultaneously measured coronary blood flow by the nitrous oxide technic increased. The direct relation of increase in velocity with increase in flow would suggest that all rather than part of the coronary vascular bed is functioning at rest and simply dilates in response to demand.

Effect of Sodium Lactate and Potassium Chloride on Electrocardiogram and Exercise Tolerance in Coronary Disease.

By *Richard S. Gubner and Donald J. Behr.* Department of Medicine, Kings County Hospital, and the Bureau of Medical Research, Equitable Life Assurance Society of the United States, New York.

Situations which may produce abnormalities of the ST segment and accompanying T wave changes, such as ischemia, digitalis, physical stress, glucose-insulin, etc., share in common the effect of lowering myocardial potassium. In order to test the hypothesis that potassium may exert a determining influence on ST segmental and T wave abnormalities, studies have been carried out on the influence of acute electrolyte changes on the electrocardiogram. Acute lowering of potassium was produced by Bellet's technic of rapid infusion of 120 cc. of molar sodium lactate within a five minute period, and elevation of potassium was produced by administering 30 mEq. of potassium in 500 cc. of an infusion given within 20 minutes.

In patients with angina pectoris whose control electrocardiograms were normal, infusion of sodium lactate produced depression of the ST segment and T wave changes similar to those occurring following performance of the two step exercise test. When exercise was carried out immediately after infusion of sodium lactate, the ST and T wave abnormalities were markedly accentuated. Conversely, exercise performed after infusion of potassium chloride resulted in lesser ST and T wave changes than exercise alone. Prior administration of potassium improved exercise tolerance and lessened anginal pain on exercise. In patients with acute or chronic myocardial disease who exhibited ST and T wave abnormalities, these were accentuated by sodium lactate and decreased by potassium infusion. Administration of sodium lactate to normal controls produced no changes other than minimal lowering of T waves.

It is suggested that myocardial and serum potassium have a determining influence on ST and T wave abnormalities, and that cardiac pain, such as occurs in ischemia, may be related to acute lowering of myocardial potassium. Chronic ST and T wave changes, as in left ventricular strain, may reflect a chronic potassium deficit of the area of the myocardium where work load is greatest. Infusion of sodium lactate may be diagnostically employed to elicit evidence of ischemia when exercise tests cannot be performed.

An Exercise Tolerance Curve—A Clinical Estimation of Myocardial Efficiency

By *G. H. Heidorn.* Altoona, Pennsylvania

The objective estimation of left ventricular function requires complex equipment usually not available to the clinician. Common carotid arterial pulse waves are being used as the basis for an exercise tolerance test which shows promise of ease of performance and fidelity in measuring left ventricular function. This is a preliminary report of the procedure and the results obtained in normal individuals.

Common carotid arterial pulse waves are

recorded by a Boucke-Brecht sphygmograph system attached to an electrocardiographic machine. The subjects undergo a standard exercise (Master). Pulse waves are obtained before and immediately after exercise as well as at 1, 2, 4, 6, 8, and 10 minute intervals following exercise. The economy of effort index (Wiggers and Katz) is calculated for each interval. A graphic exercise tolerance curve is drawn.

30 clinically normal males, a majority over 40 years of age, have been tested. The exercise tolerance curve showed a marked rise immediately after exercise with a gradual decline upon rest in all but 2 subjects who had an initially high economy of effort index at rest. An equal group of patients who had prior myocardial infarctions failed to show this sharp post-exercise rise in a majority of instances.

Pulse pressure has been closely correlated with work of the heart (Starr). The economy of effort index in normal individuals correlates very closely with the pulse pressure. However, this close correlation does not exist in many subjects with diseased hearts where exercise causes an abnormal pulse wave contour. This exercise tolerance test is now being applied to larger numbers of normal individuals as well as those with a variety of cardiovascular diseases in order to assess its value.

Observations on the Mechanism and Treatment of Shock Following Myocardial Infarction

By *R. W. Gunton, W. Paul and C. R. Woolf.* Department of Medicine, University of Toronto, and Medical Service, Toronto General Hospital, Toronto.

Using an ear oximeter and the Hamilton dye (T-1824) dilution method, hemodynamic measurements have been made in 35 patients within 24–48 hours of acute myocardial infarction and compared with 60 hospital patients without heart disease. Of the patients with infarction, 20 had no clinical evidence of shock or heart failure, 6 had hypotension (systolic B.P. 90 mm. Hg or lower) without other clinical evidence of shock, 9 had severe cardiogenic shock with profuse sweating, small or impalpable pulses, cool cyanosed extremities, weak heart sounds and clouding of consciousness.

Circulation time was prolonged in the patients with shock. Slight reduction in plasma volume with hemoconcentration was observed in all three groups with infarction. It could not have been important in the genesis of shock because it was present to an equal degree in the infarction cases without shock.

"Central" blood volume calculated from the Newman formula was increased in the patients with shock compared with the control group but was not as greatly increased as in a group of patients in left ventricular failure without myocardial infarction.

Severe reduction in cardiac output was the outstanding hemodynamic disturbance in the patients with shock. Mean values for cardiac index for the groups were: controls 3.54, infarction without shock or heart failure 3.0, infarction with hypotension 2.6, infarction with shock 1.5 L./min./M^2. The flow measurements in the patients with hypotension but without shock indicate only moderate reduction in cardiac output. These patients have a relatively favorable prognosis and may be distinguished clinically from those with shock. Evaluation of any treatment method which included this group as cardiogenic shock would err in favor of that treatment.

Forty-two patients with severe shock following myocardial infarction have been treated. Intra-arterial transfusion and vasopressor drugs have not effected any reduction in mortality when comparison is made with a concurrent series treated routinely without any special measures to restore blood pressure.

Clinical and Pathologic Studies of Anginal Pain in Congenital Cardiac Lesions Affecting the Right Ventricle.

By *Richard P. Lasser and Gabriel Genkins.* Department of Cardiology, Mount Sinai Hospital, New York.

A number of patients with congenital cardiac lesions affecting the right ventricle reported that they had experienced chest pain of an anginal nature. The clinical features of the pain patterns consisted of (1) constricting sensation in the mid- and upper chest region, caused by severe effort and relieved by rest; (2) paroxysms of severe anterior chest pain of protracted duration which was not necessarily precipitated by exertion nor promptly relieved by rest.

Cardiac catheterization studies in 9 cases revealed the presence of marked right ventricular hypertension as a common denominator in all cases. The lesions were identified as: isolated pulmonic stenosis in 5 patients, 1 tetralogy of Fallot, 1 with patent ductus arteriosus and reversed flow, 1 with interventricular septal defect and pulmonic hypertension, 1 with transposition of the great vessels.

Electrocardiograms showed pronounced right ventricular hypertrophy patterns with ST segment and T wave changes. Exercise tests reproduced chest constriction and some alteration of ST segments in 2 cases.

Postmortem examination in 3 cases demonstrated that the hypertrophied right ventricle showed the presence of scattered areas of myocardial fibrosis whereas the left ventricle did not. The appearance was that generally considered to indicate the existence of myocardial ischemia. Coronary

vessels were stated to be patent throughout, though a certain amount of atherosclerosis was found even in young individuals. The occurrence of myocardial fibrosis and its localization to the right ventricle suggested that the anginal pain was due to right ventricular ischemia secondary to the right ventricular hypertension.

The response of this pain to pulmonary valvulotomy was quite dramatic in several cases with isolated pulmonic stenosis.

An Appraisal of the Korner-Shillingford Method for Measurement of Valvular Regurgitation

By *Paul Novack, Robert C. Schlant, Florence W. Haynes, Arthur O. Phinney, Jr. and Lewis Dexter.* Departments of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston.

Indicator-dilution curves (77) from patients without valvular regurgitation have been analyzed according to the method of Korner and Shillingford (K-S). Injection sites included left atrium (LA) (30), pulmonary artery (36), and right atrium (11). A calculated central volume (CV) of less than 1.00 L. was a criterion for inclusion in this series in order that the results might be subsequently applicable to curves obtained by LA injection. The mean and standard deviations for the parameters analyzed were: aortic flow (AO) = 4.9 ± 1.9 L./min.; CV = 0.74 ± .20 L., and reciprocal of the downslope, 1/s = 13.4 ± 1.0 sec. The regression equation derived was: log 1/s = 1.7336 − 1.2256 log AO + .6708 log CV. The multiple correlation coefficient was 0.93. The standard error for prediction of slope from this equation was ±22%. As pointed out by K-S, the observed 1/s exceeds the predicted 1/s when insufficiency is present beyond the injection site. In models, K-S demonstrated that regurgitant flow (RF) could be calculated by the relationship: $RF = AO\left(\frac{s \text{ predicted } (s_p)}{s \text{ observed } (s_0)} - 1\right)$. In 15 patients with predominant mitral stenosis in whom LA curves were obtained, $s_p/s_0 = 2.52$ (1.52–3.79). The use of the values for RF so derived allows one to make interesting calculations, although the absolute accuracy of such figures remains to be proved in man. This objection, however, does not detract from the usefulness of the s_p/s_0 ratio in the detection of clinically important regurgitation.

Detection and Localization of Left-to-Right Cardiac Shunts by Indicator Dilution Curves Following Left Heart Injections

By *Eugene Braunwald, Herbert L. Tanenbaum, R. Robinson Baker and Andrew G. Morrow.* National Heart Institute, Bethesda, Maryland.

Conventional diagnostic methods often fail to precisely localize left-to-right intra- or extracardiac shunts. A technic has been developed which makes such localization possible and also permits the detection of valvular insufficiency. It consists of injecting an indicator dye into the catheterized left heart and aorta, and obtaining dye dilution curves from the femoral artery with a continuously recording densitometer.

In 29 patients without shunts or valvular insufficiency, the injection of indicator dye into the left atrium, left ventricle, or thoracic aorta yielded dilution curves with a smooth, rapid ascent and descent. In 19 patients with known left-to-right shunts, when injections were made from areas proximal to the shunt, a fraction of indicator passed through the pulmonary circulation and in all cases distinctly interrupted the smooth, rapidly descending limb of the primary curve, resulting in a secondary peak. In contrast, injections made distal to the shunt yielded normal curves. In 18 patients with valvular regurgitation, dye injections immediately distal to the insufficient valve resulted in curves with a normal rapid ascent, but a smooth, markedly prolonged descent. These results were confirmed in normal dogs, and in a group of dogs with a variety of experimentally produced left-to-right shunts and with valvular insufficiency.

The technic described has proved particularly useful in differentiating (1) uncomplicated ostium secundum atrial defect from atrio-ventricularies communis, catheter patent foramen ovale, anomalous pulmonary venous drainage, and from interventricular septal defect; (2) patent ductus arteriosus from aortic septal defect and from ruptured aneurysm of the sinus of Valsalva.

Measurement of Valvular Insufficiency by an Indicator-Dilution Method

By *W. R. Milnor.* The Johns Hopkins Hospital, Baltimore.

Insufficiency of a heart valve can be detected by injecting dye or other indicator into the chamber immediately distal to the valve and determining the amount of indicator regurgitated into the proximal chamber. If the amount injected is known, the time-concentration curve in the proximal chamber determined by continuous sampling, and the net forward cardiac output measured by an arterial time-concentration curve, the regurgitant flow can be described by a mathematical expression derived from the conventional Stewart-Hamilton formula for the calculation of cardiac output.

The *minimum* regurgitation which must be present can be calculated from the equation R = FA/M, where R = the fraction of the ventricular end-diastolic volume regurgitated with each stroke, F = net forward flow or cardiac output, A = the integrated area of the time-concentration curve in the proximal chamber, and M = amount of dye injected. The actual regurgitant volume cannot be

calculated unless atrial and ventricular volumes are known.

Tests in models showed that the regurgitation estimated by this method averaged 94% (standard error = 2.8%) of the correct value in 26 experiments. In 6 experiments on dogs with normal mitral valves an estimated mitral regurgitation ranging from 0 to 4% of ventricular end-diastolic volume was observed. In 5 experiments on dogs with surgically produced mitral insufficiency, regurgitation was estimated at 54% to 89%. In 2 patients with severe mitral stenosis and little or no mitral insufficiency as judged clinically and surgically, 5 out of 6 tests during thoracotomy showed no regurgitation of dye into the atrium, while 1 test showed 7% regurgitation.

Study of Mitral Regurgitant Blood Flow in Subjects With Normal and Deformed Mitral Valves

By *H. L. Conn, Jr., D. F. Heiman, J. C. Wood, B. Jumbala and W. S. Blakemore.* Departments of Medicine and Surgery, University of Pennsylvania School of Medicine, Philadelphia.

Surgical correction of mitral valvular disease has stimulated interest in determining the circumstances under which mitral insufficiency occurs and also in the development of better methods for detecting and quantifying it. The present study was directed toward ascertaining (1) whether normal mitral valves allow regurgitation, either in the presence or absence of atrial fibrillation, and (2) how much regurgitation occurs with deformed valves and how well the degree can be determined. Studies were made on 10 dogs and 4 humans, with and without atrial fibrillation, having normal mitral valves or having rheumatic or surgically produced mitral insufficiency. Radiopotassium was injected into the left ventricular cavity and indicator-dilution curves were obtained by measuring radiopotassium concentration with respect to time in blood from the left atrial cavity and the femoral artery. Chamber pressures were measured. Regurgitant flow was calculated from the formula:

$$\frac{\text{Left Atrial Curve Area}}{\text{Arterial Curve Area}} = \frac{\text{Regurgitant Flow}}{\text{Regurgitant + Forward Flow}}$$

Other flow and volume parameters were calculated according to the Stewart-Hamilton formula. Those subjects with normal mitral valves, irrespective of the presence of atrial fibrillation, had no demonstrable mitral insufficiency, with one exception. This occurred in one animal which developed ventricular fibrillation immediately after K^{42} injection. In the presence of mitral insufficiency calculations indicated regurgitant flow from 2% to 140% of systemic cardiac output. The apparent inapplicability of available hydraulic formulas prevented a satisfactory evaluation of mitral valve orifice area during systole. Calculations for the orifice area during diastole were likewise not feasible in the presence of insufficiency only, because of the negligible diastolic pressure gradients. Contrariwise, although there is no other quantitative method which could be applied as a standard for comparison of regurgitant flow, this dilution technic gives accurate estimations of the amount of regurgitation, at least to the extent that surgical and postmortem evaluations can serve as reference standards.

Intracardiac Phonocardiography in Man

By *David H. Lewis, George W. Deitz, Ali Ertugrul, John D. Wallace and James R. Brown, Jr.* Division of Cardiology, Philadelphia General Hospital, Philadelphia, and the U. S. Naval Air Development Center, Johnsville, Pennsylvania.

Using a miniature underwater microphone at the end of a specially designed catheter, we have recorded heart sounds from within the heart in man. This technic is virtually identical with that of underwater listening developed by the Navy for undersea warfare. The transducer is a hollow, circular cylinder of activated barium titanate, a piezoelectric ceramic. Several types of catheters have been used which allow for either sound determinations alone or simultaneous sound and pressure measurements. All of the studies have been carried out at the time of routine cardiac catheterization. Photographic records have been made of these sounds as well as tape recordings with sound spectrographic analysis.

Results indicate that the heart sounds are heard well in the lesser circulation. The first sound is loudest in the right ventricle. The second sound is loudest in the pulmonary artery. The third sound, which was not heard in most of our cases, when present was loudest in the right ventricle. The fourth sound, which was heard in all cases except those with atrial fibrillation, was loudest in the right atrium. There is, routinely, a mid-systolic murmur in the pulmonary artery even when none is heard externally. In addition to the differences in the over-all intensity of the sounds, differences have been noted in the intensity of the components of the sounds depending on the location of the catheter tip.

In certain cases of congenital heart disease the character of the murmur heard within the heart has been of value in evaluating the lesion. For example, in patent ductus arteriosus, the machinery murmur was heard in the pulmonary artery and not in the right ventricle. In ventricular septal defect, the murmur was loudest in the right ventricle in the area of the defect.

Phonocardiographic Features of Tight Mitral Stenosis

By *Thomas Leo and Herbert Hultgren.* Department of Medicine, Stanford University School of Medicine, San Francisco.

To examine the distinctive phonocardiographic features of tight mitral stenosis and to determine the diagnostic value of the technic, phonocardiograms of 21 patients were compared with auscultatory findings. All diagnoses were surgically proven. A Sanborn Twinbeam phonocardiograph was employed and simultaneous electrocardiograms, apex impulses and carotid pulses were recorded. The following observations were made:

The first heart sound may consist of distinct vibrations due to tricuspid and mitral valve closure and a pulmonary ejection sound. Sounds of tricuspid closure, identified in 12 patients, followed the QRS onset by .04 to .07 seconds. These sounds were frequently accentuated but rarely delayed after very short diastoles. Sounds of mitral valve closure followed the QRS onset by .08 to .12 seconds. They were not louder but were further delayed following very short diastoles. Pulmonary ejection sounds occurred in 13 patients, 9 of whom had severe pulmonary hypertension. These sounds followed mitral valve closure, were loudest after long diastoles, and occasionally initiated short murmurs.

Measurements of the intervals from Q to first sound therefore may be erroneous unless the mitral component of the first sound is correctly identified. Since the sound of mitral closure was equally delayed in patients with combined mitral stenosis and insufficiency, a long interval from Q to mitral closure was of no value in excluding such lesions.

The following characteristics, followed by the proportion of patients exhibiting them, were found to be reliable in identifying tight mitral stenosis: Loud sound of mitral closure (19/21); faint or absent mitral systolic murmur (19/21); opening snap, audible (13/21); recorded (18/21); absent early diastolic gallop (21/21); initiation of diastolic murmur by opening snap, recorded (16/21).

These features of tight mitral stenosis are distinctive and, when present in combination, exclude significant associated mitral insufficiency. They can be detected clinically but phonocardiography improves accuracy.

Value of Left Heart Catheterization (LHC) in Diagnosis and Treatment of Patients with Rheumatic Heart Disease

By *Joseph Uricchio, Janet Dickens, Lamberto Bentivoglio and Harry Goldberg.* Department of Medicine and The Brith Sholom Laboratory, Hahnemann Medical College and Hospital, and the Bailey Thoracic Clinic, Philadelphia.

Diagnostic and therapeutic problems arise in patients with rheumatic heart disease despite the use of available clinical data, including electrocardiographic and roentgenographic studies. The deficiencies of these conventional aids have become more apparent with attempts to define with preciseness the physiologic significance of mitral and aortic valvular lesions. The need for exactness in charting the clinical course of the rheumatic patient has been a natural consequence of the development of better surgical methods for treating acquired heart disease.

LHC allows for the measurement of pressure gradient across the mitral and aortic valves. When this data is recorded simultaneously with estimation of the cardiac output it becomes possible to obtain an accurate measurement of the degree of obstruction. Thus, LHC contributes to the necessity of surgical intervention. Furthermore, it serves as an objective method of evaluating the results of surgery.

The present report is based upon the findings in 589 patients of whom 225 had right heart catheterizations simultaneously. The following problems encountered in the diagnosis in mitral valve disease were resolved by LHC:

(1) Significance of mitral stenosis when combined with equivocal symptoms, normal ECG and roentgenogram; (2) physical signs of mitral stenosis lacking or point to a different lesion while symptoms, x-rays, and often ECG are suggestive of mitral obstruction; (3) clinical features suggest combination of mitral stenosis and regurgitation; (4) marked cardiac enlargement raising issue of whether myocardial or obstructive factor is the more significant; (5) reappearance of symptoms after a period of clinical improvement raising the question of restenosis.

In aortic stenosis LHC has helped in the following problems:

(1) To determine the dynamic significance of an aortic murmur in a patient with an equivocal history and normal sized heart; (2) angina, in a patient with a small heart and an aortic systolic murmur, due to either aortic stenosis or coronary artery disease; (3) the degree of obstruction when murmurs of stenosis, and regurgitation are present; (4) establishment of aortic obstruction in the presence of mitral disease.

The Relationship of the Diameter of the Pulmonary Artery to its Pressures, to its Resistance, to the Left Atrial Volume, to the Size of the Heart and of the Aorta and to Age of Persons with Rheumatic Heart Disease and Mitral Stenosis

By *Jacob Zatuchni, George E. Mark, Herbert M. Stauffer and Louis A. Soloff.* Departments of Medicine and Radiology, Temple University School of Medicine and Hospital. (Supported in part by a grant from the Heart Association of Southeastern Pennsylvania.)

A study was made of 25 consecutive persons with rheumatic heart disease and mitral stenosis by the combined technic of cardiac catheterization and biplane stereoscopic venous angiocardiography to determine the relationship of the diameter of the pulmonary artery to its pressures, its resistances, to

the left atrial volume, to the size of the heart and of the aorta and to age.

The diameter of the aorta varied from 19 to 45 mm. and that of the pulmonary artery from 28 to 50 mm. The diameter of the pulmonary artery exceeded that of the aorta in 20 of 25 subjects. The diameter of the pulmonary artery showed no correlation with left atrial volume ($r = 0.19$, $p = 0.64$), a low grade one with heart size ($r = 0.317$, $p = 0.88$) and with age ($r = 0.387$, $p = 0.95$), and a fair correlation with total pulmonary resistance ($r = 0.65$, $p = 0.96$), with pulmonary vascular resistance ($r = 0.55$, $p = 0.90$) and with mean pulmonary capillary venous pressure ($r = 0.51$, $p = 0.99$). The relationship was better with the difference between the mean pulmonary artery and capillary venous pressures ($r = 0.722$, $p = 0.99$) and best with the mean pulmonary artery pressure alone ($r = 0.65$, $p = 0.99$).

It is concluded that in rheumatic heart disease with mitral stenosis, the aorta is usually decreased in size relative to that of the pulmonary artery and that the increase in size of the pulmonary artery is related best to the mean pulmonary artery pressure and not at all to left atrial volume. Although higher pressures are seen with larger pulmonary arteries, nevertheless for any given size of the pulmonary artery, one may expect a wide variation of values of mean pulmonary artery pressures (Sigma = 10.7).

The Evaluation of Aortic Valvuloplasty by Left Heart Catheterization

By *Robert C. Schlant, Dwight E. Harken, Paul Novack, Florence W. Haynes, Arthur O. Phinney, Jr. and Lewis Dexter.* Departments of Medicine and Sugery, Peter Bent Brigham Hospital and Harvard Medical School, Boston.

Six patients with severe aortic stenosis have been studied by left heart catheterization pre- and postoperatively. Three patients were considered to have been significantly improved by aortic valvuloplasty on the basis of the following changes of preoperative to postoperative values: calculated aortic valve areas (AVA) of 0.4, 0.5, and 0.4 cm.^{2} increased to 0.6, 0.9, and 0.9 respectively; aortic valve mean systolic gradients (AVG) of 107, 119, and 48 mm. Hg decreased to 48, 46, and 20 respectively; cardiac indices (CI) of 6.4, 8.6, and 3.3 L./min./M.^{2} were not significantly changed; and left ventricular end-diastolic pressures (LV_d) of 20, 32, and 9 mm. Hg became 50, 21, and 11, respectively. None of these patients showed postoperative evidence of aortic insufficiency. In one case, essentially no changes resulted from surgery, the pre- and postoperative values being: AVA 0.4 and 0.5; AVG 92 and 81; CI 3.9 and 3.6; and LV_d 7 and 1. Two patients, who were apparently made worse by the creation of aortic insufficiency at operation, revealed the following changes: AVA of 0.5 and 0.4 became incalculable postoperatively because of aortic insufficiency; AVG of 85 and 31 decreased to 60 and 0 respectively; CI of 5.2 and 2.0 became 2.0 and 2.5 respectively; and LV_d of 10 and 8 increased to 20 and 11 respectively. This study suggests that (1) the degree to which a calcified, stenotic aortic valve has been enlarged has been limited because of the likelihood of substituting insufficiency for stenosis, but that (2) even small increments in AVA have resulted in significant reductions in the gradient of pressure across the aortic valve; and (3) this is an extremely useful method in suggesting alterations in technic and improvement in surgical approach.

A New Concept in Prosthetic Material For Use in Plastic Cardiac Surgery

By *Goffredo G. Gensini, Vicente N. Roger, Robert L. Hawley and Gardner Middlebrook.* Cardiopulmonary Laboratory, Department of Research and Laboratories, The National Jewish Hospital, Denver.

No ideal prosthetic substance for plastic cardiovascular surgery has yet been found. A material specially prepared from sheep caecum and used for the manufacture of condoms was selected for investigation. This paper-thin laminate membrane was chosen because of its mammalian origin, pliability and strength.

This investigation was undertaken to determine the extent to which this substance could be functionally incorporated into vascular tissues. The experimental animals used were dogs and this material was grafted into arteries, veins and the heart. Semilunar valves were constructed in aortas.

At first, leukocytes and fibrin appeared in the spaces between the lamina. Then fibroblasts migrated from the host tissue through the spaces, utilizing the laminae as a framework. Within two months the fibrotic replacement was complete. The host tissue showed no foreign reaction to the prosthesis. The semilunar valves retained original shape with free edges, open sinuses and appeared to have increased strength. The leaflets were slightly but uniformly thickened and somewhat smaller. Thrombotic phenomena did not occur on the heart grafts or on the valves. However, the pulmonary artery grafts became thrombosed.

The rate of fibrosis of the prosthesis appears to be adequate to insure retention of the graft. This is probably due to guiding of the proliferating fibroblasts through the interstices of the laminae resulting in a laminated structure with great strength.

The concept of a cardiovascular prosthesis being completely replaced by multi-layered fibrous connective tissue may represent an improvement over previously used irreplaceable, nonlaminated substances.

In conclusion, a new material is proposed that

seems to possess superior qualities and offers the opportunity of constructing artificial cardiac valves. This tissue, derived from sheep caecum, is pliable, adaptable, compatible with the host tissue, and allows for rapid and complete replacement by strong, fibrous connective tissue of the host.

A Correlation of Electrocardiographic and Hemodynamic Changes Following Closure of Atrial Septal Defects

By *Leonard S. Dreifus, Sheldon Bender, Janet Dickens, Daniel F. Downing and Harry Goldberg.* Departments of Medicine and Pediatrics and the Brith Sholom Cardio-pulmonary Laboratory, Hahnemann Medical College and Hospital, and the Bailey Thoracic Clinic, Philadelphia.

Surgical closure of atrial septal defects afforded a unique opportunity to observe the electrocardiographic changes which occur following the correction of the intra-cardiac shunt. Seventy-six patients with complete closure of their defect were selected for this study. Electrocardiographic changes were observed in both the early (immediate to 1 month) and later postoperative periods (2–3 years).

Preoperatively, the typical rSR′ configuration was present in 60% of cases and qR in 19%. In the remainder an R or rR′ was observed with notching on the upstroke of the R. In 2 cases a normal ECG was present. If a qR was present the typical rSR′ was seen in V-2 and the initial r in V-1 was considered isoelectric. There was no significant correlation of QRS duration or height of R wave in V-1 with right ventricular pressure. Electrocardiograms with QRS durations of 0.06–0.11 sec. show no correlation with the flow index (PF/SF), but the largest flow index was associated with QRS of 0.12 sec. or greater.

Postoperative catheterization studies performed in 39 patients revealed closure of the defect in all. A decrease in the height of the positive deflections R or R′ in V-1 and S in V-6 with narrowing of the QRS was evident within 2 to 3 weeks following closure of the shunt. Narrowing of QRS duration was seen even with those patients demonstrating normal right ventricular pressure prior to surgery. Follow-up ECG's up to 3 years later did not reveal any further significant decrease in QRS duration or QRS configuration except that R in V-1 and S in V-6 tended to become further reduced in amplitude. In almost every instance there was a definite change in P wave configuration following closure of the defect with a lowering in lead 2, 3, V-1, and V-2 even though the P waves appeared within normal limits prior to surgery. Although P wave configuration was altered following anatomic closure of the defect, disturbances in sino-auricular pacemaker activity were not frequently encountered (as contrasted to mitral valvular disease where left auricular pressures are usually increased) in spite of P wave abnormalities. Complete or incomplete right bundle branch system block is apparently secondary to the anatomic lesion, and appears irreversible after closure of the shunt or decrease in right ventricular pressure.

The Question of Myocardial Failure in Patients with Mitral Stenosis and Low Cardiac Output

By *Thomas Killip III and Daniel S. Lukas.* Department of Medicine and Cardio-Pulmonary Laboratory, New York Hospital-Cornell Medical Center, New York. (Aided by grants from the National Heart Institute and New York Heart Association.)

The absence of significant pulmonary arterial hypertension in patients with mitral stenosis and low cardiac output has been interpreted as evidence that myocardial failure rather than mitral obstruction is the major physiologic lesion. It has been suggested that such patients will not benefit from mitral valvuloplasty.

In a series of 260 patients with mitral stenosis studied by cardiac catheterization 9 such patients (all with atrial fibrillation) were found. The hemodynamic pattern consisted of a cardiac index half normal, slightly increased pulmonary arterial pressure, pulmonary "capillary" pressure < 15 mm. Hg and pulmonary vascular resistance < 375 dynes/sec. cm.$^{-5}$ Five patients had resting oxygen consumptions < 110 ml./min./M.^{2}, which accounted for a part of the reduction of output. During exercise cardiac output increased in all and PA and "PC" pressures rose markedly. Increase in output appropriate for the degree of exercise in 6 despite the increased pressure load was regarded as evidence against myocardial insufficiency. The calculated mitral valve orifice was < 0.7 cm.^{2}/M.^{2} B.S.A. in all and fingertip stenosis was encountered in 7 who underwent surgery. Preoperatively, acute digitalization in one did not increase cardiac output; one year postoperatively, cardiac index was 50% higher and pulmonary vascular pressures during exercise were half preoperative levels. One patient who did not have valvuloplasty subsequently developed acute pulmonary edema under emotional stress.

The data are interpreted as indicating that significant mitral obstruction may exist despite low cardiac output and pulmonary vascular pressures at rest and may be brought to light by studies during exercise and relation of flow and "PC" pressure to valve area. Failure to improve following operation in such patients should be interpreted with regard to the degree of residual stenosis as well as the level of myocardial function

The Acute Effects of Simultaneously Increasing Central Venous Pressure and Peripheral Resistance on Cardiac Output

By *Robert H. Eich, Ingolf Staib, and Maurice Wertheimer.* State University of New York, Upstate Medical Center, Syracuse.

To investigate the hemodynamic effects of simultaneously increasing peripheral resistance and central venous pressure, normal subjects were studied before and after the inflation of a standard Air Force antigravity suit. At rest, in supine patients, the suit has been shown by other workers to increase central venous pressure (CVP) and peripheral resistance in the legs, while producing only a transient elevation of blood pressure which rapidly returns to control levels. In addition, in normal subjects, we have not been able to demonstrate a consistent change in finger flow following inflation.

The acute effects of inflation on cardiac output, central blood volume, CVP, and blood pressure, were studied in 10 normal subjects. The dye dilution technic using iodinated (I^{131}) human serum albumin was used for the measurement of cardiac output and central blood volume. The subjects were studied supine and the suit was inflated to 80 mm. Hg. In spite of a mean increase in CVP of 7 cm. H_2O, the mean cardiac index fell from 3.58 to 3.43 L./min./$M.^2$ In those with a BSA over 1.9 $M.^2$ there was a somewhat larger fall in cardiac index, and a more marked increase in calculated total resistance increasing from 152 to 380 dynes $cm.^{-5}$ sec. This is a function of the tightness of the suit to start with. There was no significant change in blood pressure, and no consistent change in central blood volume, although the suit does displace blood into the lungs.

These findings appear to offer more evidence in favor of the concepts that the heart can take an active part in blood pressure regulation, and that the cardiac output can vary independently of central venous pressure in maintaining a constant blood pressure level.

The Effect of Hydrocortisone on Urinary Sodium Excretion in Patients with Congestive Heart Failure

By *Robert G. Page and A. R. Lavender*. Department of Medicine, The University of Chicago, Chicago.
(Aided by a grant-in-aid from the American Medical Association.)

Patients with congestive heart failure who were in fair control were studied on the metabolic floor. Total balance of water, sodium, potassium, chloride, and nitrogen were measured. After a suitable control period, the patients were given 160 mg. of hydrocortisone for 16 days and then allowed to recover. The response of urinary sodium excretion varied and tended to divide the patients into three groups. The first type of response was shown in a patient with little edema whose weight decreased during the time of hydrocortisone administration, and whose urinary sodium excretion showed a slight increase. The second type was demonstrated in three moderately edematous patients who showed either little change or a gain in weight during hydrocortisone administration, this being accompanied by a diminished urinary sodium output. Following discontinuance of the drug there was a sharp increase in urinary sodium output accompanied by weight loss. The final type was demonstrated in a patient whose daily urinary output of sodium was approximately 3 mEq. during the control period. There was no change in this patient's urinary sodium either during hydrocortisone administration or after its discontinuance.

A Study of Osmole and Water Excretion in Congestive Heart Failure

By *Norton Spritz, George W. Frimpter, Warren S. Braveman, and Albert L. Rubin*. Second (Cornell) Medical Division, Bellevue Hospital Center, and Department of Medicine, Cornell University Medical College, New York.

The osmole to water relationships during mercurial-induced diuresis have been studied by others in normal human subjects and animals. To determine the applicability of these findings to the diuresis obtained in patients with edema, we made similar measurements in six patients with congestive heart failure.

In fasting patients who had received no mercurial for several days, a mercurial diuretic was given to each and urine specimens were collected in fifteen minute periods. Three subjects received Pitressin intravenously, either throughout the study or after the establishment of diuresis. Urine flow, plasma and urine osmolality were determined, and from these data osmolar clearances and free water reabsorption were calculated.

A linear relationship was obtained between osmolar clearance and rate of urine flow during diuresis, just as observed during mercurial diuresis in normal individuals and experimental animals. This relationship of solute to water excretion is consistent with the concept that a mercurial diuresis is an osmotic diuresis, that free water reabsorption is relatively fixed, and that total water excretion is equal to the osmolar clearance minus this fixed amount of free water reabsorbed.

As Pitressin administration did not affect free water reabsorption in these subjects, it would appear that endogenous antidiuretic activity is maximal under these experimental conditions.

In these patients some variation in free water reabsorption did occur, unrelated to change in endogenous creatinine clearance or to plasma osmolality. In contrast to the results of studies carried out in normal human subjects, these variations did not relate to the phase of mercurial diuresis.

In these subjects, the capacity for free water reabsorption—one factor affecting the ratio of water to solute excretion during mercurial diuresis—was significantly lower than that which had been reported in normal subjects. This may be related to renal tubular functional alterations secondary to congestive heart failure.

The use of Multiple Drug Therapy in the Management of Severe Benign and Malignant Hypertension

By *Fred T. Darvill, Jr. and John L. Bakke.* Veterans Administration Hospital and the Department of Medicine, University of Washington School of Medicine, Seattle.

To study the possible advantages of combined use of three to five antihypertensive drugs, 12 patients with severe benign hypertension and 10 patients with malignant hypertension were treated for 3–51 months (average 18 months).

Reserpine was administered first, and Dibenzyline fifth; ganglionic blocking agents, hydralazine, and veratrum preparations were prescribed in variable order depending on clinical indications. The initial drug was administered alone, and dosage increased until the average therapeutic dose was reached, or until side effects became disabling; if blood pressure (BP) control (average diastolic pressure of 100 mm. Hg or less) had not occurred, another drug was added. This process was repeated until BP control was achieved or until all 5 drugs were being administered. All patients reported received at least 3 drugs.

Clinical evaluation, funduscopic examination, electrocardiogram, chest x-ray, PSP excretion, urinalysis, BUN, and IVP were done initially and at least yearly thereafter; Regitine was administered initially. Home BP graphs were obtained continuously to determine therapeutic response and regulate dosage.

Diastolic BP reduction was 10–20 mm. Hg in 4 patients, 20–40 mm. Hg in 14 patients, and over 40 mm. Hg in 4 patients. Retinopathy improved in 12 patients; heart size diminished, and electrocardiographic abnormalities improved in 8 patients; renal function improved in 3 patients. The only parameter exhibiting deterioration was renal function (4 patients). Thirteen patients returned to employment. Three patients died, one with probable pulmonary infarction, and two, initially uremic, of progressive renal failure.

In 5 instances, the addition of a fourth or fifth drug resulted in a BP reduction not previously obtainable; conversely, the gradual withdrawal of any one of several drugs from the therapeutic regimen resulted in a significant diastolic BP rise which could again be controlled by resumption of the drug.

Side effects seemed less severe and patient acceptance better using these methods.

Chemical Control of Cardiac Work in Congestive Heart Failure

By *William E. Huckabee.* Evans Memorial of Massachusetts Memorial Hospitals.

It appears at present that any biochemical abnormality which will explain human myocardial failure must account for all the clinically observed pharmacologic and physiologic influences, or at least respond in the same way. The stimulus response characteristic of the human myocardium under demand for increased work output has not been described, since the basic stimulus is not known. Cardiac filling pressure is considered unlikely to be the controlling stimulus because of its lack of correlation with output in human subjects. Investigation of chemical control of the circulation, however, has yielded striking correlations.

Twenty normal subjects and 26 patients with heart failure, of severity from Class II to IV, were mildly exercised. Cardiac index (or stroke volume) increase (ΔC.O.) in either group was not correlated with change in QO_2, mixed venous blood oxygen content or tension, pCO_2, pH, $[K^+]$, hematocrit, lactate, pyruvate, ketoglutarate, citrate or glucose concentrations. Blood epinephrine and norepinephrine concentrations fell, usually to zero. However, from these data tissue anaerobic metabolic rates were calculated (rate of energy derivation from LDH system in substitution for oxygen). They showed a close correlation with ΔC.O. in normals ($r = .77$) and also in the variable cardiac group ($r = .75$). Slopes were 79 ml./min.ΔC.O./ml. O_2/min. AMR, in normals, and significantly lower in cardiacs, 9 ml./min./ml./min., despite a similar mean ΔC.O. Chemical control by direct action on the myocardium (by rH) cannot be established. Normals showed a y-intercept of 2074 ml./min., a "superimposed" ΔC.O. due to some other factor (i.e., pressure) which was completely absent in the cardiacs. All the failing hearts exhibited the same response curve; it was not affected by therapy and clinical improvement. The patients, therefore, appeared to differ in clinical severity because of different peripheral phenomena. This myocardial characteristic of human patients is consistent with the concept of a permanently abnormal cardiac myosin in congestive failure.

A Method for Serial Cardiac Output Determinations Applied to the Study of Pregnancy Associated with Heart Disease

By *Robert W. Cornett and James Metcalfe.* Departments of Medicine and Obstetrics, Harvard Medical School, and the Medical Clinic, Boston Lying-in Hospital, Boston.

The technic of cardiac catheterization is for practical purposes inapplicable to making numerous serial cardiac output determinations in individual patients.

An alternative method, which is applicable to serial use, consists of using the lungs as an aerotonometer and CO_2 as the indicator gas. The pCO_2 of end-expiratory gas is measured during normal quiet breathing. This represents alveolar and arterial pCO_2. A volume of gas is then equilibrated with mixed venous blood by rebreathing, and its pCO_2

measured. The derived A-V difference of CO_2 tension is converted to A-V difference of CO_2 content. With the measurement of CO_2 production, pulmonary blood flow can be calculated.

The lack of data concerning the cardiovascular adjustments to pregnancy in women with heart disease led the authors to make use of this method for determining cardiac output serially and at frequent intervals in a group of pregnant women with rheumatic heart disease.

Over 100 cardiac output determinations were made during pregnancy and the postpartum period on 11 women with rheumatic heart disease. Control studies were made on normal nonpregnant subjects, and on pregnant women without heart disease.

The variability of this measurement in nonpregnant control subjects was less than ±10% of the mean. Comparable consistency of results was found between consecutive cardiac output determination in pregnant subjects, during the period of pregnancy when cardiac output is known to change only gradually.

In all pregnant subjects studied there was an increase in cardiac output reaching its peak during the third trimester, and decreasing before parturition. The mean increase was less in pregnant cardiacs than in pregnant normals.

It is concluded that, because of the reproduceability of results and ease of application, the method described is useful for making serial determinations of cardiac output.

Experimental Studies on Ventricular Gallop Rhythm

By *Arnold M. Weissler, James J. Leonard and James V. Warren.* Department of Medicine, Duke University, Durham, North Carolina.

The temporal association of the ventricular gallop and the phase of rapid ventricular filling has served to date as the major evidence for the cause and effect relationship of these phenomena. In an attempt to investigate this relationship more fully, the effects of simple venous occlusive tourniquets on all extremities were studied in 15 cases with ventricular gallop rhythm. Simultaneous logarithmic phonocardiograms and apex cardiograms were recorded before, during and after five minutes of blood pressure cuff inflation to diastolic levels.

In the presence of ventricular gallop rhythm, large rapid deflections were noted on the ventricular diastolic filling wave in the apex cardiogram. Application of cuffs completely obliterated the ventricular gallop sound in ten of the 15 cases. In all such cases where undistorted apex cardiograms could be recorded, there was simultaneous diminution in the slope of the ventricular filling wave and disappearance of the rapid gallop deflections. Release of cuffs in these individuals resulted in prompt reappearance of the gallop sound with return of the more rapid filling wave and the gallop deflection.

Further observations have been made on the intracardiac pressure phenomena associated with gallop rhythm. Ventricular pressure curves in individuals with gallop rhythm often demonstrate positive diastolic pressure waves coincident with the gallop sound. Simultaneous recordings of right atrial and ventricular pressures through a double-lumen catheter with synchronized frequency tested strain gauges have revealed actual transient reversals of the atrioventricular pressure gradient at the time of the gallop sound in individuals with right-sided gallop rhythm.

These data lend support to the view that (1) gallop phenomena are indeed causally related to rapid ventricular filling and that (2) atrioventricular valve tensing or reclosure may be the cause of the gallop sound.

A Pressor Mechanism Associated with the Postpartum State

By *Frank A. Finnerty, Jr., and Joachim H. Buchholz.* Department of Medicine, Georgetown University School of Medicine and the Georgetown and George Washington Obstetrical Division, District of Columbia General Hospital, Washington, D. C.

Recent experience in a toxemia clinic has shown that the period 2 to 20 weeks postpartum is frequently associated with asymptomatic hypertension. Serial studies on 1706 patients have shown the sequence of an entirely normal past history, prenatal course, delivery, and immediate postpartum course with the first occurrence of an elevated arterial pressure at 2 to 7 weeks postpartum in 133 patients. Although multiparity predominated (average, 5 pregnancies), the average age of the group was 21 years. Mean arterial pressure increased from an average of 92 mm. Hg to 135 mm. Hg, a 46% average increase. Physical examination and routine laboratory studies including careful ophthalmoscopic examination, x-ray of the chest, urinalysis, and ECG revealed no other evidence of vascular disease.

Hospitalization in 15 patients (including 72 hours bed rest) was associated with only a 10% decrease in mean arterial pressure. Response to ice, tilting, methanesulfonate, and histamine was not abnormal. Cardiac output and renal blood flow determinations were normal. The average duration of the hypertension was 12 weeks (7 to 42 weeks).

That some as yet undefined pressor mechanism exists during the postpartum period seems probable, since (1) serial studies on 1130 hypertensive patients (history of hypertension prior to pregnancy) have shown the highest level of arterial pressure recorded during this period in 79 patients; (2) studies on patients followed through multiple pregnancies have shown the recurrence of transitory hypertension only during the postpartum period in 30 patients; and (3) studies on 4 patients with transitory hypertension

recorded only postpartum currently show persistent hypertension.

Although the exact pressor mechanism responsible for postpartum hypertension remains unknown, it would seem that anxiety or stress do not play a major role, since labor and delivery are not associated with even transitory elevations of arterial pressure in these patients. It would seem further that transitory postpartum hypertension may initiate the course of hypertensive vascular disease in at least some patients.

The Incidence of Pyelonephritis in "Essential" Hypertension

By *C. M. Smythe, C. F. Rivers, R. M. Rosemond, and F. N. McCorkle.* Department of Medicine and the Cardiac Clinic, Medical College of South Carolina, Charleston.

Thirty-nine hypertensive patients, 37 of whom were Negroes, have been carefully studied for occult pyelonephritis. All had diastolic hypertension of 110 mm. Hg or over. Patients adequately screened for renal disease and those with known pyelonephritis were excluded from this study. Included were either recently discovered hypertensives or patients followed in the medical out-patient clinic as essential hypertensives without known pyuria.

Three either clean voided (males) or catheterized (females) urine specimens for smear, culture, colony count, and urinalysis were obtained from all patients. Twenty-eight patients had less than 10^3 organisms/ml. in all uncontaminated specimens. Six patients had more than 10^5 organisms/ml. in all specimens and two others had more than 10^5 in two of three specimens. Three patients had the same organism constantly present in counts of 10^2 to 10^5 organisms/ml. Therefore, eight patients had significant bacilluria, and three more probably significant bacilluria. Of these eleven, nine had intravenous pyelograms, five of which showed poor function and only one a contracted kidney. Also only one of these 11 had constant pyuria and two had intermittent pyuria.

Thus pyelonephritis occurred in 28% of these so-called essential hypertensives. Significant bacilluria frequently exists in the absence of pyuria. So many routine urine cultures are positive and the presence of pyuria so variable that colony counts are of greatest value in the detection of significant bacilluria. This study would indicate that counts of greater than 10^5 organisms/ml. are definitely significant, counts of less than 10^4 organisms/ml. are not significant, and counts of 10^4 organisms/ml. may or may not be significant.

In this high incidence of pyelonephritis may lie one of the answers to the frequency and resistance to therapy of hypertension among Negroes.

Studies on Oxygen Saturation in Superficial Veins of the Forearm

By *Solbert Permutt and Marian Isaacs.* Medical Division, Montefiore Hospital, New York.

Multiple blood samples obtained through indwelling Cournand needles in superficial veins on the volar surface of the forearm in nine normal subjects had consistently high oxygen saturations. The average of 68 samples was 89.1% (S.D. 4%) (in contrast to much lower and more variable saturations in blood simultaneously obtained from deep forearm veins). Saturations in blood from superficial veins of four hyperthyroid subjects were not significantly different. Three patients in congestive heart failure showed significantly lower saturations. When hand circulation was occluded by a wrist cuff at 250 mm. Hg in normal and cardiac subjects, oxygen saturation fell an average of 17%; but saturations in hyperthyroid subjects were either unchanged, or more often increased. In normal subjects, mild leg exercise produced no change unless hand flow was occluded prior to exercise, and then a significant further reduction in saturation occurred during exercise. Strenuous exercise to the point of exhaustion always produced marked reduction in saturation, even without hand flow occlusion. Cardiac patients exhibited this marked reduction following very mild leg exercise. During mild exercise hyperthyroid patients showed an intermediate response, with some reduction in saturation; but strenuous exercise had no additional effect. Therefore, under the latter conditions, hyperthyroid subjects had much higher saturations than normal subjects.

Evidence will be presented which suggests that the superficial veins of the forearm drain mainly the hand and skin of the forearm when the hand flow is intact and the skin of the forearm alone when hand flow is occluded. If this premise is correct, the above data can be utilized in making certain deductions regarding the control of blood flow to these areas in health and disease.

The Effects of Intravenous Infusions of Epinephrine and Norepinephrine on Their Plasma Concentrations

By *Hugo Garcia and John M. Wallace.* Department of Medicine, Duke University, Durham, North Carolina.

Norepinephrine (NE) and epinephrine (E) were measured by modified ethylenediamine method. Intravenous infusions of NE and E were given to 18 subjects. Ten received between 3 and 19 μg./min. of NE and 8 received 1.5 to 22.0 μg./min. of E. Intra-arterial pressure was recorded and simultaneous peripheral venous (V) and arterial (A) samples withdrawn. Changes in NE of 1.00 μg./L. and

in E of 0.20 μg./L. were significantly beyond differences between duplicates.

NE infusions: The average preinfusion NE A-V difference was negative, −0.36 μg./L., that during infusion was positive, +1.00 μg./L. Three and 4 μg./min. produced little rise in plasma concentrations; 6 to 12 μg./min. increased levels; 17 to 19 μg./min. caused no further increases. Mean values were an increase of 3.4 μg./L. at a rate of 11.4 μg./min. Average mean arterial pressure rose 18 mm. Hg and pulse decreased 12 beats. Following infusion, NE A-V differences became negative within the first postinfusion minute and arterial levels were baseline in 5 to 10 minutes.

E infusions: The average preinfusion E A-V difference was zero, that during infusion was positive, +0.46 μg./L. One and one-half to 3.0 μg./min. caused variable increases in plasma levels and only small further rises were accomplished by larger amounts. Mean values were an increase of 1.03 μg./L. at a rate of 6.0 μg./min. Mean arterial pressure rose zero mm. Hg and the pulse increased 11 beats. Following infusion, E A-V differences were negative the first 5 minutes and arterial levels were baseline in 3 to 5 minutes.

At these infusion rates, in both groups, plasma levels of the corresponding amine did not rise after the first 1 to 3 minutes. Postinfusion return of blood pressure and pulse to normal preceded by 2 to 3 minutes return of arterial concentrations to baseline. Symptoms did not correlate well with plasma levels.

A Fully Automatic Graphic Method to Study Circulation Time and Vascular Sufficiency by Means of Fluorescein (Dermofluorography)

By *M. M. Mahl, K. Lange and L. J. Boyd.* Department of Medicine, New York Medical College—Metropolitan Medical Center, New York (Vascular Research Group, Bird S. Coler Hospital).

A new fully automatic recording method of determining circulation time and vascular sufficiency by means of fluorescein was developed. The Dermofluorograph consists of a photocell rigidly aligned to a long-wave ultra-violet light source; the output of the photocell is electronically amplified and recorded on a strip chart recording galvanometer. The time elapsing from the intravenous injection of a fluorescein solution to its arrival in the face or extremity (arm to face or arm to leg circulation time) can be determined fully automatically. At the same time, the steepness of the resulting curve, after arrival of the dye, mirrors the blood supply of the skin, as can be shown experimentally. The test may be performed on unconscious patients, and in infants and small children with or without congenital heart disease. The instrument is portable and the test is performed at the bedside, without the necessity for special rooms or precautions. The tests may be repeated at three minute intervals; the results are reproducible with a maximal deviation of ±5%.

In over 100 cases, a comparison study of the circulation time determined with decholin to this fully automatic objective circulation time shows that decholin circulation times are highly unreliable, especially in patients with failure. It appears that large residual cardiac blood volumes tend to increase the error of the subjective method.

The arm to leg times of a large number of normals and of patients with occlusive vascular disease were studied. In individuals with occlusive vascular disease, the circulation time to the extremity was prolonged and/or the resulting curve was much shallower than normal. The method gave information as to the vascular status of the skin of the extremity, especially regarding the development of collateral circulation, information which could often not be obtained by other methods.

The Effect of Serotonin Upon Systemic Small and Large Vessel Resistances

By *F. J. Haddy, M. Fleishman, D. A. Emanuel and J. B. Scott.* U. S. Army Medical Research Laboratory, Ft. Knox, Kentucky.

The effect of 5-hydroxytryptamine upon artery, small vessel and vein caliber has been studied in the forelegs of 21 pentobarbitalized dogs. These effects were compared in the same animals with those produced by epinephrine and norepinephrine. Foreleg blood flow rate was maintained constant with a blood pump while pressures were measured directly in the brachial artery, foot pad small artery (0.5 mm.), subcutaneous small paw vein (0.5 mm.) and cephalic vein utilizing fine glass tubes. Serotonin creatinine sulfate, 4.6 γ base/min., epinephrine hydrochloride, 2.1 γ base/min. and l-norepinephrine bitartrate, 2.5 γ base/min. were separately infused into the brachial artery over 5 minute periods. Serotonin had little effect upon brachial artery and cephalic vein pressures, greatly decreased small artery pressure and sometimes increased small vein pressure (maximal level 32 mm. Hg). Epinephrine and norepinephrine greatly elevated brachial and small artery pressures. The mean segmental resistance changes ± S.D. during serotonin infusion were arteries +0.77 ± 0.37, small vessels −0.71 ± 0.33, veins +0.08 ± 0.15 and total +0.10 ± 0.41 mm. Hg/ml./min. The corresponding changes during epinephrine and norepinephrine infusion were −0.14 ± 0.20, +1.25 ± 0.56, −0.02 ± 0.05, +1.12 ± 0.54 and +0.16 ± 0.23, +1.33 ± 0.41, +0.07 ± 0.10, +1.56 ± 0.56, respectively. These findings appear to demonstrate that serotonin causes pronounced constriction of large arteries and sometimes large veins concomitant with dilatation of small vessels (probably arterioles). In contrast, epinephrine and norepinephrine primarily constrict small vessels. The

directionally opposite changes in segmental resistances account for the failure of serotonin to greatly effect total resistance and offer further evidence that arteries, small vessels and veins may actively function as independent resistances. The results may explain how serotonin produces peculiar skin color changes without predictable blood pressure variations in patients with carcinoid tumor and when injected intravenously into intact animals or man. They also suggest that serotonin may influence volume distribution of blood within various segments of the circulatory system, cardiac filling, and water and salt distribution across capillary membranes.

The Role of the Sympathetic Nervous System in the Responses of Blood Flow to Skin and Muscle

By *A. Bogdanovics, W. Redisch, K. DeCrinis and J. M. Steele.* Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, Welfare Island, New York.

Blood flow to the lower extremity was measured under controlled conditions in young adults, elderly adults without demonstrable vascular disease, hemiplegic patients, paraplegic patients, and patients with occlusive peripheral arterial disease before and after sympathectomy. Their responses to body warming, body cooling and exercise were studied. Separate estimations were made of skin flow and muscle flow.

Basal flows were lower in elderly adults than in young adults, but there was no essential difference in responses to physiologic and pharmacologic stimuli, except that increase in muscle flow following exercise was considerably less than in young adults. Patients with obliterative arterial disease had low basal flows and delayed responses. Sympathectomy and cord dissection above T_1 produced high basal flows and materially altered responses to vasomotive stimuli. Central nervous system lesions not affecting the autonomic system did not alter vasomotive responses. From these data it can be concluded that neurogenic vasomotor responses are mediated only through the autonomic pathways and that, at least in the extremity, blood flow to the skin is more dependent upon neurogenic regulation than blood flow to the muscle.

Effects of Heparin and Dicumarol on Atherogenesis in Cholesterol-Fed Cockerels

By *Jeremiah Stamler, Ruth Pick and Louis N. Katz.* (With the assistance of Dolores Friedman and Philip Johnson.) Cardiovascular Department, Medical Research Institute, Michael Reese Hospital, Chicago.

In view of the growing utilization of heparin and Dicumarol in the long term treatment of human atherosclerotic disease, the effects of these drugs were assessed on experimental atherogenesis in cholesterol-fed cockerels.

Three series of experiments were accomplished using depot heparin (2, 10 and 20 mg. /bird /day) in chicks ingesting mash supplemented with 0.5, 1.0 and 2.0% cholesterol plus 5% cottonseed oil. Heparin did not suppress diet-induced hypercholesterolemia, nor did it lower levels of any β-lipoproteins classes in intact, alloxanized or depancreatized cockerels. Coronary and aorta atherogenesis were also unaffected.

In two series of experiments with oral Dicumarol (4 to 30 mg./bird /day), aggravation of cholesterol-induced coronary atherosclerosis occurred; no effects were observed on hyperlipemia or aorta atherogenesis. In one experiment, heparin and Dicumarol counteracted the ability of estrogens to prevent coronary lesions, i.e., both anticoagulants apparently exerted a positive atherogenic effect.

Despite prolongation of clotting time, no evidence was obtained of hemorrhage into plaques.

These findings suggest that heparin and Dicumarol—in the presence of the prerequisite dietary and lipid metabolic alteration—may potentiate atherogenesis in the chick and that this possibility must be considered in man.

An Estimated Probability For An Arteriosclerotic Unilateral Leg Amputee To Become A Bilateral Case

By *Sung J. Liao and Arthur L. Watkins.* Commission on Chronically Ill, Rocky Hill, Connecticut, and Bay State Medical Rehabilitation Clinic, Massachusetts General Hospital, and Harvard Medical School, Boston.

With the aging of the population in this country, chronic diseases, especially those due to degenerative processes, have assumed increasing importance. Arteriosclerotic gangrene of lower extremities is one of the major causes of prolonged disability, because amputation of one limb or both may often be resorted to. Frequently the patient will ask whether his other leg will have a similar fate and, if so, how soon. In a recent study of bilateral leg amputees with regard to their rehabilitation potentialities, we have accumulated some data relevant to this question. It is our purpose to present the results, hoping to stimulate further critical studies of a similar nature.

During the past 6½ years, 192 lower extremity amputees (due to arteriosclerotic vascular insufficiency alone) were admitted to Bay State Medical Rehabilitation Clinic, Boston, Massachusetts. Thirty-three of them (17%) were bilateral amputees. This latter group had similar age and sex distribution as that of the unilateral cases. They were mainly elderly individuals with an average age of about 63 years at the time of their first definitive amputation. From two weeks to 15 years later, these 33 patients underwent amputation of the other leg. Fifty per

cent of them had the second definitive surgery at the end of six months. Only 13 of these 33 used a prosthesis before they had to lose the other leg. Fifty per cent of the 13 had this operation in 2¼ years with a range of five months to 15 years. Of the 20 who did not use any prosthesis 50% had the last operation around the end of three months. One of many plausible reasons for this was that those who could use a limb during the interim were probably in better physical condition to tolerate the hardships of using it. Six of those who never had an opportunity to use it had to have the additional amputation within three months. Nevertheless, this does not exclude the possibility that an artificial limb does contribute, to some extent, a reduction of weight bearing on the remaining leg, causing less demand on its peripheral vascular system. Further studies of this aspect are certainly imperative.

Conclusion: The probability for a unilateral amputee (due to arteriosclerotic changes) to lose the other leg may be as high as 17%. While a prosthesis may seem to protect the remaining "good" leg, further investigation is required.

Splanchnic Blood Volume in Congestive Heart Failure

By *Elliot Rapaport, Myron H. Weisbart and Milton LeVine.* Medical Service, V. A. Hospital and the Department of Medicine, Albany Medical College, Albany, New York.

Most investigators have demonstrated that total blood volume in congestive heart failure is increased. An expanded cardiopulmonary volume has been implicated as one primary factor responsible. The purpose of the present study was to determine whether splanchnic blood volume is increased in congestive failure, and if so, its proportional influence in the production of an increased total blood volume.

Splanchnic plasma volume (T-1824 space) was measured in 12 patients with moderate to severe congestive heart failure, and splanchnic blood volume was approximated by use of the large vessel hematocrit. The results were compared with similar studies in 10 normal patients.

Mean values in control patients were as follows: Estimated hepatic blood flow 813 cc./min./M.^{2} (s.d. = 102); ratio of estimated hepatic blood flow to cardiac output 25.7% (s.d. = 7.7); splanchnic oxygen uptake 41.7 cc./M.^{2} (s.d. = 11.0); splanchnic blood volume 12.7 cc./Kg. (s.d. = 2.2); total blood volume 62.1 cc./Kg. (s.d. = 7.0); ratio of splanchnic blood volume to total blood volume 20.8% (s.d. = 5.1%). Mean values in the congestive heart failure patients were: Estimated hepatic blood flow 674 cc./min./M.^{2} (s.d. = 257); ratio of estimated hepatic blood flow to cardiac output 32.8% (s.d. = 10.3); splanchnic oxygen uptake 42.6 cc./M.^{2} (s.d. = 4.3); splanchnic blood volume 20.8 cc./Kg. (s.d. = 5.6); total blood volume 79.8 cc./Kg. (s.d. = 12.5); ratio of splanchnic blood volume to total blood volume 26.5% (s.d. = 7.4).

The results indicate that splanchnic blood volume is significantly increased and furnishes a major contribution to the increased total blood volume observed in congestive heart failure, despite the fact that splanchnic blood flow is frequently reduced. It would appear, therefore, that the splanchnic blood volume is not solely regulated by splanchnic blood flow but that splanchnic pooling may be caused by an increased venous pressure acting on a relatively elastic capillary-venous reservoir.

The Effect of Induced Cardiac Acceleration Upon the Coronary Hemodynamics and Cardiac Metabolism of the Intact Anesthetized Dog

By *G. M. Maxwell, C. A. Castillo, G. G. Rowe, D. H. White, Jr. and C. W. Crumpton.* Cardiovascular Laboratory, University of Wisconsin, Madison.

Intact dogs were anesthetized with morphine and pentobarbital; control cardiac output and coronary flow (method of Kety and Schmidt) were then estimated. The heart was then electrically stimulated by means of a catheter in the right atrium so that a definite increase (at least 80%) in heart rate was obtained. The cardiac output and coronary flow was repeated. Nine dogs were satisfactorily studied. The animals were then sacrificed and left ventricular weight was estimated.

The results showed that general systemic hemodynamics and metabolism remained essentially unchanged with increase in heart rate. However, the coronary flow rose significantly (from 81 to 148 cc./100 Gm. myoc./min.); the arterial/coronary sinus oxygen difference remained the same. Thus, the cardiac metabolic rate for oxygen rose as a function of increased flow; the cardiac metabolic rate for carbon dioxide was similarly affected. The coronary vascular resistance fell significantly; the flow per beat was unchanged. The coronary blood flow—left ventricular work ratio rose significantly. The index of efficiency fell.

The calculated absolute myocardial oxygen consumption rose, while the mechanical efficiency of the heart fell markedly.

EDUCATION

A Preliminary Report on the Evaluation of Clinical Teachers

By *Nicholas J. Cotsonas, Jr., Harry F. Dowling, and Robert J. Kaiser.* University of Illinois College of Medicine, Chicago.

We have attempted an evaluation of clinical teachers through the use of two student questionnaires—one structured, the other unstructured. The latter is completed in an exercise devoted to clerical evaluation at the end of each semester. The student is free to comment on any aspect of his teacher's performance he chooses.

The structured questionnaire, which has been devised in collaboration with the College of Education, has three advantages: it obtains specific answers to individual features that are considered to be characteristic of good clinical teaching; the answers can be measured quantitatively; and it contains 35 questions in six categories: the personal qualities of the teacher, his teaching technics, the teaching session, the effectiveness of teaching by example, the teacher's knowledge, and an over-all value judgment on the teacher. The entire class completes this questionnaire at one time at the end of the year.

Both questionnaires are utilized in large group discussions which include all the teachers in the program and in which each instructor is handed cards containing a summary of the data concerning him. The results of this questionnaire have been compared with the rankings made independently by three coordinators who know these teachers best.

The Goals of a Psychiatric Consultant in a "Comprehensive Care" Teaching Program

By *Peter T. Janulis, and Albert J. Stunkard.* Comprehensive Care Program, New York Hospital—Cornell Medical Center, New York.

An analysis of difficulties encountered by medical students in dealing with the emotional problems of their medical patients allows for a formulation of goals for a "Comprehensive Care" teaching program, such as that at Cornell University Medical College.

In a setting of comprehensive medical care, problems of (1) psychiatric diagnosis and (2) management of "difficult" patients, such as those who are hostile, uncooperative, etc., frequently confront the student. As would be expected, an even more common problem is a patient whose symptoms are essentially the manifestations of a relatively acute "neurotic" reaction to a current life situation. Not uncommonly these symptoms will subside rapidly after a negative physical examination and reassurance by the physician. If they persist, however, the student may have a number of typical difficulties: (1) difficulty recognizing the presence of neurotic conflict; (2) the excess use of reassurance and support; (3) the premature giving of advice; (4) difficulty in getting the patient to see the nature of his problem; (5) difficulty proceeding with brief psychotherapy once the nature of the problem has become apparent to the patient; and (6) setting a realistic goal.

Factors contributing to such difficulties include (1) the student's primarily medical orientation, (2) his inexperience, (3) his failure to recognize the underlying "problem" as being the patient's "neurotic" reaction and not some external situation, (4) his difficulty in conceiving an adequate approach to this problem and (5) his misapplication of psychiatric knowledge already in his possession. All too frequently the result is an unclear formulation which hinders the student's therapeutic efforts and leads to his discouragement and frustration.

One important teaching goal, therefore, is to help the student to cope adequately and realistically with such "neurotic" reactions. He should:

1. Recognize the symptoms as manifestations of neurotic conflict.

2. If possible, help the patient become aware of the relationships between symptoms, emotional reactions and underlying character traits and attitudes.

3. If possible, in a joint effort with the patient, consider approaches to shifting the balance of forces in the patient's current interpersonal situation through modification of attitudes, greater self-assertion or environmental manipulation.

4. Set a flexible, realistic goal for his therapeutic efforts, such as helping the patient through the acute period, and consider the patient's need and motivation for further psychotherapy.

The Psychotherapy Study Group: A Method of Postgraduate Education in Psychotherapeutic Aspects of Medical Practice

By *Albert Stunkard and Peter Janulis.* Study Program in Human Health and the Ecology of Man, Departments of Medicine and Psychiatry, New York Hospital—Cornell Medical Center, New York.

Two years ago a small group of internists in the general medical clinic of the New York Hospital requested a short course on psychiatric methods which might help them in dealing with the emotional problems of their patients. In discussion with the psychiatric consultants it was decided that this need might best be met by the formation of a psychotherapy study group which would attempt to make explicit those aspects of the practice of medicine which involve the doctor-patient relationship. The meetings of this group were stenographically recorded, and these records have been carefully analyzed.

The focus of each meeting was on a particular problem faced by one of the members of the group.

No effort was made to teach any theory of personality functioning or to extend considerations of theory beyond the needs of the problem at hand. Instead, major emphasis was placed upon the creation of an atmosphere of respect for the efforts of other members of the group, and trust in their good will. In the atmosphere which developed, the participants were able to discuss with remarkable honesty what occurred in their treatment of patients. The resultant increased awareness of many aspects of treatment stimulated the members of the group to explore ways of establishing more meaningful and more helpful relationships with their patients. The support of their colleagues helped them to master the anxiety aroused by these efforts.

Despite the limited expectations of its founders, the study group has continued to meet weekly for a period of two years and has attracted new members until one fourth of the clinic staff of 60 physicians is now in regular attendance. Most of the members report increased interest and satisfaction in treating the emotional problems of their patients; their case presentations provide evidence that this treatment is more effective.

The Professional Educator: Enemy or Friend?

By *George E. Miller*. University of Buffalo, Buffalo.

The professional educator is widely regarded as an impractical pedant more intent upon the methodology of instruction than its substance. This stereotype has led to the stony rejection of his potential contribution to education in medicine.

The Project in Medical Education at the University of Buffalo attempts to bridge this chasm of hostility and scorn. During the 1956–57 academic year professionals in education have conducted a continuing series of seminars and workshops for four local medical faculty members and three visiting representatives of other medical schools, each of whom carries a teaching load in this school. Illustrative examples extracted from two of five topic areas may serve to convey the nature and some of the outcome of this study.

Evaluation. An analysis of current practice in evaluation of students leads inevitably to the conclusion that medical faculties commonly overlook (1) the purpose of the appraisal, (2) the criteria for the appraisal, (3) whether the device measures what we want to know, and (4) whether it is in keeping with over-all educational objectives. Answers to these questions provide a helpful reorientation to the potential contribution of meaningful evaluation to student progress.

Instructional Methods. The development of a medical course outline commonly begins with the arbitrary assignment of hours to laboratory, clinic, seminar or conference, the lecture presently suffering an eclipse. When the goals of instruction are first defined, however, it is quickly apparent that some of these avenues may be useless, and that even the lecture may fill an important need. Once goals have been identified a new horizon of methodology suggests itself.

This experience suggests that the professional educator may be far more practical, far less concerned with methods, far more concerned with instructional substance, than we.

Educational Responsibility and the Hospital

By *Robert I. McClaughry*. Providence Hospital, Detroit.

The hospital collects groups of sick persons together for the treatment of these patients' ills. These groups also account for centering the study of illness in the hospital. These dual functions of patient care and study have recurrently presented serious problems. In each instance, a significant renewal in medical progress has followed the solution of such problems.

The present status of graduate medical education emphasizes such a problem; it exists also in other phases of clinical study. The physician's chief interest is in the care of patients. This has naturally resulted in the activities of the physician in training centering in the technical aspects of medical care. Alan Gregg has spoken of this as concern with "what to think," as contrasted with "how to think." Maximum benefit from his hospital experience is thus thought to result if the resident physician is constantly busy taking care of hospitalized patients.

This pragmatic approach has proved insufficient. An external system of evaluating hospital educational programs has supplemented this for many years. Accreditation of hospitals by the Council on Medical Education and Hospitals of the American Medical Association, and examination of qualified candidates by the American Boards in the medical specialties constitute this valuable external evaluation.

There remains, however, an important area of educational responsibility requiring development. Each educational institution must be independently responsible for its standards in three areas: admission to study, satisfactory completion of a prescribed program, and certification of such completion. Many accredited hospitals must still take steps toward assuming such responsibility. When this is accomplished, fear and distrust of the external examining agencies can be expected to be replaced with deserved respect.

A Method for Evaluating Student-Patient Interviews

By *Guy Hollifield, C. T. Rousell, A. J. Bachrach and E. G. Pattishall*. School of Medicine, University of Virginia, Charlottesville.

History taking is one of the physician's basic methods of investigation. It is axiomatic that a

medical history must be accurate and must be obtained by methods which will enlist the patient's cooperation. Efforts to develop interviewing skill in medical students are hampered by lack of methods which permit evaluation of this skill and hence evaluation of programs designed to develop it.

A team composed of an internist, a social worker and two psychologists has characterized a medical student-patient interview by the following traits: initiation, ease of interviewer, ease of patient, control, pertinence, transition, termination and transmission. A five point evaluation scale was devised for each of these traits. This team then evaluated 210 new patient interviews tape recorded by 68 medical students. These tape recorded interviews were identified by a code unknown to the evaluating team. From two to six months after the initial evaluation, a stratified random sample of 30 was reevaluated by the same team without reference to the initial scores. A comparison of these scores with the initial ones produced an over-all coefficient of correlation of .888. For the individual traits the coefficients of correlation were: initiation .51, ease of interviewer .55, control .75, pertinence .78, transition .81, termination .80, transmission .74. (Values significant at .01 level when coefficient of correlation equal .46.)

This method of evaluating student-patient interviews proved to be a highly reliable one and is suitable for measuring changes in performance.

The One-way Screen as an Adjunct to Clinical Teaching

By *Clinton G. Weiman and George G. Reader.*

Teaching medical students to communicate effectively with patients is an important part of clinical teaching. Considerable effort is usually expended in teaching students interviewing technic, for example, through use of manuals outlining procedure and itemizing the facts to be obtained in the history. By the end of the fourth year many students are still not equipped to obtain a medical history in an efficient and precise manner in spite of manuals, directions by instructors and experience with a large number of patients.

A one-way vision screen is not new as a method of observing doctors and patients; but as an instrument for teaching interviewing to medical students, it has been found to afford a unique advantage to the instructor. The teaching of second year students at Cornell was modified only to the extent of providing a demonstration with the one-way screen in addition to the usual lectures and practice sessions with gratifying results.

Small groups (8–10) of students were observed through the one-way screen as one of their colleagues interviewed a patient. The instructor with the group pointed out various aspects of the interview situation and then a critique was held when the interview was concluded. Frequently the performance of inexperienced second year students who had learned in this way excelled that of senior students trained with an interview manual.

Some educators believe first and second year students cannot learn to interview patients because they lack information on which to base intelligent questions. The findings of this study utilizing the one-way screen technic would tend to refute such opinions.

ENDOCRINES AND METABOLISM

Clinical Application of Densitometric Technics in Measurement of Body Fat

By *Edward J. Werdein and Laurence H. Kyle.* Department of Medicine, Georgetown University Medical Center, Washington, D. C.

Body composition can be assessed by either densitometric or volume distribution technics. This report concerns evaluation of these 2 methods used singly, in combination, and in conjunction with nitrogen balance for the measurement of total body fat. Body density was obtained by underwater weighing 161 times in 45 subjects. Total body water (TBW) was measured by volume distribution of antipyrine, radioantipyrine or D_2O 124 times in 57 subjects. Nitrogen balance was followed in 14 of the studies. The results are analyzed regarding feasibility, reproducibility and accuracy as concerns both estimation of fat content and change in total body fat.

Although rigorous, underwater weighing has proved practical in normal subjects and has been satisfactorily accomplished in a wide variety of metabolic disorders. Reproducibility is good and correction for residual lung volume permits measurement to within 1.5 Kg. of fat. Use of total body water is much simpler, readily reproducible and applicable when underwater weighing is impossible. Both technics are distorted by hydration abnormality. This can be circumvented in part by simultaneous measurement of density and body water. The accuracy of both technics rests on the assumption that the fat-free body (FFB) has a known and constant density; calculation of % water in the FFB indicates that this assumption may be incorrect. When *change* in fat is studied by repeated measurements, the possibility of abnormal FFB density is much less important but hydration abnormality introduces significant error. Consequently, neither technic of fat estimation is reliable if used singly.

Application of both methods, with correction of hydration abnormality is much more satisfactory, and concurrent nitrogen balance study adds further reliability.

The studies indicate that total body fat can be estimated by either densitometric or volume distribution technics, but that application of both methods is more reliable. These technics of fat estimation are particularly effective when coupled with balance study.

Nitrogen Balance in Obese Patients After Prolonged Weight Reduction on Low-Calorie, Low-Protein Formula Diets

By *Alvan R. Feinstein and Irving L. Schwartz.* Hospital of the Rockefeller Institute for Medical Research, New York.

Few studies of nitrogen balance have been reported after prolonged weight reduction of obese patients. Strang, McClugage and Evans (1931), Young (1952), and Young, Ringler and Greer (1953), using relatively high protein intakes, measured 7–9 day samplings of nitrogen balance after more than 8 weeks of weight reduction in 5–10 patients. Their data indicate nitrogen balances ranging from −3.0 to +3.2 Gm./day on intakes of 357–1400 cal./day containing 8.4–16.6 Gm. N/day. They found no consistent relationship between the nitrogen intake and balance, and observed no significant physiologic or clinical correlations with periods of either positive or negative nitrogen balance during the dieting or subsequently.

The present report gives preliminary results of nitrogen balance measurements obtained as part of continuing investigation of orally-fed liquid formula mixtures as the sole source of nutrients in metabolic studies. After prolonged periods of weight reduction, 6–10 day balance samplings were done in 7 obese patients. Except for sporadic 1–14 day intervals, they had been maintained in the hospital on formula diets for periods of 3–14 mos. At certain times intake was kept high enough to maintain weight; at other times it was lowered to 590–900 cal./day, resulting in sustained weight loss.

Four patients had been on caloric restriction for 4–8 mos., with weight losses of 21.9–46.7 Kg. Of these, three were receiving 590 cal./day containing 2.13 Gm. N and showed balances of −1.2 to +0.1 Gm. N/day. The fourth, on 900 cal./day containing 3.06 Gm. N, had a balance of −0.4 Gm. N/day. Two patients on caloric restriction for 2½–3 mos., with weight losses of 21.3 and 28.2 Kg., were given 590 cal./day, containing 2.13 Gm. N, and showed balances of −1.83 and −2.25 Gm. N/day. The seventh patient, receiving formulas providing 600 cal. and 3.60 Gm. N/day had lost 38.6 Kg. in 9 mos. At this time, the N-balance was +0.12 Gm. N/day. When her N-intake was later doubled isocalorically to 7.20 Gm./day, N-balance became +0.98 Gm./day.

The nitrogen balance values observed in the present group are within the range of variation cited in the earlier studies of conventional high protein diets. The smallest negative values were noted in the five patients who had been maintained on formula reduction diets for the longest periods of time and had lost the largest amounts of weight. In one instance, isocaloric doubling of the protein intake did not alter nitrogen retention substantially.

Pyruvic Acid Metabolism in Obesity

By *Donald Berkowitz and Nathaniel Berk.* Sidney Hillman Medical Center, Philadelphia.

In an attempt to explain the pathogenesis of obesity, Pennington has recently suggested that a block in carbohydrate metabolism at the pyruvate level may be a factor.

To study this hypothesis we performed glucose and fat tolerance tests on 20 non-obese controls and 20 obese subjects. The test meals used were 100 Gm. dextrose, and a mixture of 2 egg yolks and 3 ounces of heavy cream. Blood glucose, lactic and pyruvic acid levels were determined before and at serial times after ingestion of the meal.

Following ingestion of the dextrose, the controls demonstrated a rise in pyruvic and lactic acid values. The peak occurred at one hour, with a gradual decline to the basal values by the third hour.

In the obese group, half showed a diabetic glucose tolerance curve. The maximum rise in lactic and pyruvic acid was not significantly different from the normal weight subjects. The peak value of pyruvic acid did occur later, however. In the normals this occurred between 30 and 60 minutes after the test meal, whereas in the obese group, the maximum rise was after 1 hour. Breakdown of the obese patients according to their glucose tolerance curve showed that this delay in the pyruvic acid peak was actually a function of the diabetic status, rather than the body weight.

In the studies with the fat meal, no significant difference in the blood glucose, lactic or pyruvic acid values between the two groups was evident.

Our results do not indicate any significant abnormality in pyruvate metabolism following a dextrose or fat meal in the obese patient. It is suggested that in studies with obese subjects, the presence or absence of diabetes be considered before attributing metabolic alterations to the obese state per se.

Hypophyseal Control of Fasting Ketosis in the Rat

By *Thomas T. Amatruda, Jr. and Frank L. Engel.* Departments of Medicine and Physiology, Duke University, Durham, North Carolina.

While an adenohypophyseal control of ketone metabolism has been suggested by the ketogenic

activity of adenohypophyseal extracts and the reported suppression of ketosis by hypophysectomy, the magnitude and significance of this control remain obscure. In this study the effects of a 7 day fast on glycemia and ketonemia in normal and phloridizinized rats were compared to those in recently (<60 days) and remotely (>105 days) hypophysectomized rats with and without cortisone treatment, and in hypophysectomized-adrenalectomized rats. Normal rats displayed maximal ketonemia (7.7 ± 0.54 mg.%) and minimal glycemia (76 ± 3.2 mg.%) on day 2. Thereafter, ketonemia fell to 2.5 ± 0.24 mg.% and glycemia rose to 96 ± 3.0 mg.% by day 7. In recently hypophysectomized rats ketonemia increased to a plateau of 10 to 12 mg.% on days 5–7, while glycemia declined to 41 ± 7.6 mg.% by day 7. Remotely hypophysectomized rats reached a maximal ketonemia of 19.4 ± 1.62 mg.% with glycemia of 42 ± 5.1 mg.% on day 7. Adrenalectomy did not modify the responses of remotely hypophysectomized rats, but treating them with 0.25 mg. cortisone q.d. resulted in ketonemia and glycemia simulating the normal response. Since the fasting hypophysectomized rats developed hypoglycemia, a known ketogenic stimulus, normal rats were phloridizinized for a control study. During hypoglycemia comparable to that of hypophysectomized rats, the phloridizinized normals developed much greater ketonemia (68.5 ± 5.2 mg.%). These data demonstrate that while the hypophysis is not essential for the development of ketosis, the degree of response is blunted by hypophysectomy. The adrenal cortex presumably moderates fasting ketosis in a permissive fashion by allowing gluconeogenesis to occur.

Effects of Long-Term Estrogen Therapy on Serum Lipids and Lipoproteins in Inborn Errors of Lipid Metabolism

By *Elaine T. Bossak, Chun I. Wang and David Adlersberg.* Department of Medicine, Mount Sinai Hospital, New York.

The present study is concerned with the effects of long-term treatment with ethinyl estradiol (Estinyl) on the serum lipids and lipoproteins and clinical course of patients with inborn errors of lipid metabolism. Lipids were determined chemically and lipoproteins by paper electrophoresis and subsequent staining with Oil Red O.

The study included 4 men and 2 women with idiopathic hyperlipemia, average age 44, and 3 men and 3 women with idiopathic hypercholesteremia, average age 46. Five patients had skin xanthoma and 2 had xanthoma tendinosa; 5 had coronary artery disease; 2 had acute abdominal crises suggesting pancreatitis and 1 had diabetes mellitus. The average duration of therapy was 8 months (range 1–13 months). Courses of Estinyl therapy have been replaced for several months by placebo therapy.

In both groups lipid levels were lower after estrogen therapy. Maximum effects were noted after 6 weeks. In the hyperlipemic group levels of serum total and esterified cholesterol averaged 495 mg.% and 340 mg.% before therapy and decreased, by 43% and 46%, to 280 mg.% and 183 mg.%. Total lipids fell 34% from 2379 mg.% to 1562 mg.%. Average serum phospholipids before and after therapy were 544 mg.% and 434 mg.% (a fall of 20%). Alpha-lipoprotein increased by 82% from 13% to 23.7% of the total stainable lipid. Beta-lipoprotein was unchanged (38.0% and 37.5%) and the O(Origin)-fraction decreased by 20% from 49.0% to 38.8%.

In the hypercholesteremic group levels of serum total and esterified cholesterol averaged 472 mg.% and 358 mg.% before therapy and decreased, by 38% and 39%, to 294 mg.% and 220 mg.%. Total lipids fell 21% from 1285 mg.% to 1019 mg.%. Average serum phospholipids before and after therapy were 409 mg.% and 373 mg.% (a fall of 9%). Alpha-lipoprotein increased by 44% from 19.6% to 35.2% of the total stainable lipid. Beta-lipoprotein decreased 25% from 87.0% to 50.2% and the O-fraction was unchanged (13.4%, 14.6%).

Levels of lipids and lipoproteins in patients on placebo therapy were unchanged from control levels. The initial daily dosage of Estinyl was 0.2 mg., later decreased to a minimum maintenance dose of 0.1 mg. daily. Patients were on a low-fat diet instituted prior to the inception of the study, and maintained a stable body weight. Moderate gynecomastia and diminished libido were observed uniformly in men. In 2 post-menopausal women mild uterine bleeding occurred. In some instances, if side effects were troublesome, placebo therapy was temporarily instituted and symptoms disappeared. In 2 patients the cardiac status improved while 3 were unchanged. In 1 patient with extensive xanthoma tuberosa there was gradual disappearance of the lesions during 8 months of estrogen therapy.

The Serum Lipid and Lipoprotein Response to the Administration of Androgens and Estrogens

By *Robert H. Furman, R. Palmer Howard, Leonard N. Norcia and E. Corinne Keaty.* Oklahoma Medical Research Foundation and the School of Medicine, University of Oklahoma, Oklahoma City.

The results of a four year study of the influence of gonadal steroids on serum lipids and lipoproteins in more than 60 human subjects are presented. Estrogens promptly and consistently increase high density $-S_{1.21}$ O-12 ("alpha") lipoprotein concentrations, while androgens lower them. Characteristically, lower density $-S_{1.21}$ 25–40 ("beta") or $-S_{1.21}$ 25–70 lipoprotein concentrations either do not change, or change in a direction opposite to that of

the high density lipoproteins, when gonadal steroids are administered. The response of the serum cholesterol and phospholipids, as determined chemically, may be predicted on the basis of the concentration changes induced in the two major lipoprotein fractions by gonadal steroid administration in individual subjects. When high and low density lipoprotein levels vary in opposite directions, little or no change in the concentrations of cholesterol or phospholipids in native serum occurs. When lower density lipoprotein concentrations fail to change following gonadal steroid exhibition, serum cholesterol and phospholipid levels vary in the same direction as that of the high density lipoproteins. Serum phospholipid levels tend to vary with the high density lipoprotein fraction more than the serum cholesterol levels, probably because of the relatively high phospholipid/cholesterol ratio in the $-S_{1.21}$ O-12 lipoproteins.

Lipoprotein changes induced by gonadal steroids tend to persist for one or more weeks after cessation of therapy, and after nitrogen balance has returned to the control state. No "wearing off" phenomena have been observed.

These serum lipoprotein changes induced by gonadal steroid administration suggest that the relative paucity of coronary atherosclerosis in healthy women may be attributable to either relatively large concentrations of high density lipoproteins or relatively high values for the high density/lower density lipoprotein ratios.

Inhibition of Cholesterol Absorption by Wool Fat Sterols

By *Maurice M. Best and Charles H. Duncan.* Department of Medicine, University of Louisville School of Medicine, Louisville, Kentucky.

Common characteristics of sitosterol and the other reported sterol inhibitors of cholesterol absorption are a free hydroxyl group on carbon-3 which may be esterified, and the property of being readily precipitated by digitonin. The 30 carbon sterols which occur in wool fat possess a free hydroxyl group on C-3 but they do not form a difficultly soluble digitonid. It thus seemed of interest to determine whether they would interfere with cholesterol absorption.

"Isocholesterol," a mixture of 30 carbon sterols derived from wool fat, was added to the diet of male albino rats for a two week period, both alone and in combination with cholesterol. At the end of two weeks the animals were killed and the serum and liver concentration of cholesterol determined. Mean serum cholesterol, in mg./100 ml., and mean liver cholesterol, in mg./100 Gm. liver (in parenthesis) were as follows: basic diet 72 ± 5 (303 ±6); basic diet plus 1% cholesterol 88 ± 8 (1323 ±285); basic diet plus 5% "isocholesterol" 82 ±6 (324 ± 26); basic diet plus 1% cholesterol and 5% "isocholesterol" 68 ± 5 (342 ±32). Making use of the differences in the absorption spectra produced by cholesterol and by "isocholesterol" in the Abell modification of the Lieberman-Burchardt reaction, no "isocholesterol" was detected in the livers.

The data from these and human studies indicate that "isocholesterol," while not itself absorbed in detectable amounts, exerts an inhibitory effect on the absorption of cholesterol. This result is compatible with the hypothesis that the sterol inhibitors of cholesterol absorption act by tying up the esterification mechanism of cholesterol absorption.

The Efficacy of Corn Oil in Lowering the Serum Cholesterol of Patients with Coronary Atherosclerosis

By *Louis Tobian and Naip Tuna.* Department of Medicine, University of Minnesota School of Medicine and the University of Minnesota Hospitals, Minneapolis.

Twenty-three coronary patients drank one ounce of corn oil three times daily and used it as a cooking and salad oil. Five patients were given no other diet restrictions; 18 were instructed to avoid butter fat and hydrogenated vegetable fats. Lean meat and all other foods were allowed *ad libitum.* 70% of the patients taking corn oil had a sizeable drop in serum cholesterol level, at least 15% or more. The average pretreatment cholesterol level of this group was 238 mg. %. During the corn oil regimen the cholesterol level fell to an average value of 182, a 56 mg. drop. Serum phospholipids were determined in half of this group. The average pretreatment phospholipid level was 287 mg. %. The average level fell to 231 during corn oil ingestion. Another 26% of the patients had smaller decreases of 9 to 14% in serum cholesterol while taking corn oil. The average pretreatment cholesterol level for this group was 238, while the average level during the corn oil program was 208. 30% of the patients were eating a low fat diet during the control period and still had a significant decrease in serum cholesterol after taking corn oil. The full effect on serum cholesterol could usually be seen after three weeks of corn oil feeding. Two patients have taken corn oil for 8 months and have consistently maintained low cholesterol levels. 28% of the patients with angina pectoris reported considerable subjective relief while ingesting corn oil; none became worse. Only one patient had indigestion while taking one ounce of corn oil before each meal. The iodine value of serum lipid in one patient changed from 83 during the pretreatment period to 124 after four weeks on corn oil.

The Effect of Triiodothyronine on Serum Lipids and Lipoproteins of Euthyroid and Hyperthyroid Subjects

By *Bernard A. Sachs, Ethel Danielson, Marian C. Isaacs and Raymond E. Weston.* Medical Division, Montefiore Hospital, New York.

The inverse relationship between thyroid production and the level of serum lipids has been well established. The availability of the more potent analogue of thyroxine, triiodothyronine, presented an opportunity for studying its effects on the serum lipids and lipoproteins of carefully controlled euthyroid and hyperthyroid subjects maintained on fixed food and fluid intakes on a metabolic ward. L-triiodothyronine (0.4 to 6.0 mg. daily) was administered intravenously in divided doses for 3 to 23 days to 3 normal subjects, one hyperthyroid patient during a partial spontaneous remission, and one patient with frank thyrotoxicosis. Hypermetabolism was produced in the euthyroid subjects and increased in the hyperthyroid patients.

Serum total lipids, lipid phosphorus, total cholesterol, beta-lipoproteins and protein fractionation (by either densitometry or elution) after paper electrophoresis were determined before, during, and following triiodothyronine administration. Serum lipids and lipoproteins began to decrease proportionally within 1 to 4 days of the onset of therapy. The decrease in total lipid varied from 15 to 50%, in lipid phosphorus from 19 to 56%, and in total cholesterol from 23 to 56%. Profound falls in beta-lipoprotein were also observed but changes in protein fractions were inconstant. The serum lipid partition returned to control values 7 to 15 days after cessation of therapy. The observed fall in lipids persisted after therapy longer than did the other clinical and laboratory evidences of hypermetabolism, which subsided within 4 to 6 days.

Further studies of the lipolytic effects of triiodothyronine are indicated to determine the mechanism of its action and the possible role of lower doses of this potent substance in the treatment of disorders of lipid metabolism.

Effect of a Synthetic Steroid on Serum Lipids and on Serum Protein-Bound Iodine

By *John H. Peters, A. Henry Randall and Mary G. Bell.* Veterans Administration Hospital, and Department of Medicine, Emory University School of Medicine, Atlanta.

Recently much interest has been focused on methods for reducing the concentration of various serum lipid components. Preliminary studies of 17 α-methyl-19-nortestosterone (17MNT), a steroid with both estrogenic and androgenic structural characteristics, revealed that it depressed serum cholesterol in animals. Administered to women in doses up to 300 mg./d. it has suppressed ovulation and displayed a predominantly progesterone-like action with minimal masculinizing or feminizing effects. In the present study 17MNT, in doses of 6–50 mg./d., has been administered sublingually to one normal male and to seven individuals with hypercholesterolemia of various types. Serum total cholesterol, and fractionation of lipoproteins by ultracentrifugation were performed. Effects on serum cholesterol were not consistent or of great magnitude. The majority of the subjects showed dramatic increases in the Sf 0–12 lipoproteins without consistent or significant variations in the other fractions.

Clinical observations revealed no consistent subjective response of the patients, but in three instances observers suspected alterations in thyroid function. Determination of protein-bound iodine revealed significant decreases in concentration in all but three subjects, two of whom had initial concentrations of less than 2γ%. One of the latter did not have an increase in PBI during 17MNT administration despite an increase in thyroid dosage.

The findings suggest that this steroid has a prompt and profound effect on the metabolism of the thyroid hormone. Failure of decreases in PBI to be accompanied by rises in cholesterol remain unexplained to date. Additional studies of thyroid function are under way to determine whether the observed changes are paralleled by analogous alterations in I^{131} metabolism. Should subsequent data reveal that 17MNT decreases metabolism without inducing hyperlipemia, it would have theoretical advantages over I^{131} in the therapy of angina pectoris.

(17MNT has been called variously methylnortestosterone and methandrolone in America and methyl-estrenolone in Europe.)

The Binding of Iodide by Normal Human Serum

By *Marvin L. Mitchell.* Lemuel Shattuck Hospital, Boston.

The elevation in nonthyroxine serum protein-bound iodine concentration following the prolonged ingestion of inorganic iodide has been fully described but poorly understood. Electrophoresis of serum shortly after administration of therapeutic radioiodine to patients has demonstrated association of I^{131} with serum albumin. Since a small per cent of the I^{131} added with inorganic iodide to human serum in vitro was also associated with serum albumin, further studies on the binding of I^{131} by normal human serum were carried out using an equilibrium dialysis system. I^{131} in a phosphate buffer dialyzing medium (pH 7.2–7.4) was equilibrated with a known volume of normal serum in a cellophane dialysis membrane for a period of 24 hours at 3°C. Comparison of counting rates between equal aliquots of dialyzed serum and medium revealed 1.5 to 2.5 times more activity in the serum. Paper electrophoresis and radioautography demonstrated the I^{131} to be associated mainly with the serum albumin, and approximately 75% of the bound activity could be removed from the serum with trichloroacetic

acid. The addition of compounds such as sodium thiosulfate and potassium perchlorate in concentrations greater than 0.05 M prevented binding, while sodium thiocyanate and propylthiouracil were still effective at weaker strengths. Increasing concentrations of potassium iodide in the medium, both during and following I^{131} equilibration, resulted in a progressive reduction in the I^{131} gradient between serum and medium. Sera from subjects receiving large doses of potassium iodide were subjected to paper electrophoresis. The albumin zone was eluted with physiologic saline and calculation of the stable iodine content accounted for a major fraction of the serum non-thyroxine protein-bound iodine concentration. These data suggest that the increased concentration of non-thyroxine serum protein-bound iodine following the ingestion of potassium iodide may be attributed to a relatively loose binding of iodide by serum albumin.

Observations on the Use of Thyroid Stimulating Hormone (T.S.H.) in the Differentiation of Primary from Secondary Hypothyroidism

By *David Plotkin, Charles R. Kleeman and William H. Blahd.* Department of Medicine and Radioisotope Service, Veterans Administration Center and University of California Medical Center, Los Angeles.

The R.A.I. uptake response to T.S.H. (Thytropar) has been frequently utilized, but poorly standardized, for the differentiation of primary from secondary (pituitary) hypothyroidism. To date, 22 cases (10, primary and 12, secondary) have been evaluated utilizing T.S.H. in single (10 units I.M.) or multiple doses on consecutive days.

Routine 6 and 24 hour R.A.I. uptakes will not differentiate per se primary from secondary hypothyroidism. The 6 hour uptakes (before and after T.S.H.) were of no value in differentiation. In the 10 primary hypothyroids the 24 hour uptake failed to rise more than 4 percentage points above the pre T.S.H. uptake. Of the 11 secondary hypothyroids 8 showed a rise of at least 7 percentage points above the pre T.S.H. value. Three secondary hypothyroids were completely refractory to T.S.H. The etiology or the duration of the pituitary type hypothyroidism did not seem to influence the relative sensitivity to T.S.H. stimulation. In both types of hypothyroidism a single injection of 10 units of T.S.H. appeared to be as adequate as multiple doses given over several days in eliciting a rise in R.A.I. uptake if the rise was obtainable at all.

The levels of protein-bound iodine (P.B.I.) and cholesterol in the blood were of no value in differentiating primary from secondary hypothyroidism.

The data, to date, would suggest that the response of the 24 hour R.A.I. uptake after 10 units of potent T.S.H. is an adequate differential test except in those cases of secondary hypothyroidism completely refractory to T.S.H. Multiple dose tests would seem to have little additional value. This is in contrast to the improved adrenal function after multiple doses of ACTH in subjects with secondary adrenal insufficiency.

The Effect of Thyrotropin on the Serum Pattern of Thyroid Hormones

By *Walter L. Arons and Jerrold D. Hydovitz.* Department of Medicine of the Hospital of the University of Pennsylvania, Philadelphia, and the Institute of Research, Montefiore Hospital, Pittsburgh.

Radiopaperchromatographic studies have been carried out on the peripheral serum of 8 patients following the intramuscular administration of 10 to 20 units of thyroid stimulating hormone (TSH). The TSH was injected after several hours before, or 48 to 72 hours following, the administration of a therapeutic dose of I^{131}. At varying intervals from 1 to 48 hours after thryotropin administration, chromatographic separation and characterization of thyroid hormones was achieved by the use of both one and two dimensional solvent systems. The effect of TSH on the serum patterns of thyroxine, 3:5:3′ triiodothyronine, monoiodotyrosine and diiodotyrosine has been studied in four hyperthyroid and four euthyroid individuals. In both the euthyroid and the hyperthyroid groups the administration of thyroid stimulating hormone was followed by a prompt increase (within 3 to 6 hours) of thyroxine radioactivity in the serum. In the four euthyroid subjects no change in radioactive triiodothyronine serum levels was noted except in one subject in whom an increase in triiodothyronine occurred approximately 30 hours after the administration of thyroid stimulating hormone. By contrast, three of the four hyperthyroid patients demonstrated small and transient rises in triiodothyronine levels within two to six hours after the thyrotropin had been administered. Chromatographic studies carried out before and after TSH administration did not demonstrate any additional areas of radioactivity to suggest the presence of detectable quantities of either 3:3′ diiodothyronine or 3:3′5′ triiodothyronine in the peripheral serum of these hyperthyroid and euthyroid patients. These data would indicate that the administration of thyrotropin is followed by the rapid appearance of small amounts of 3:5:3′ triiodothyronine in the serum of hyperthyroid individuals, while a similar response could not be demonstrated in the euthyroid subjects.

Genital and Systemic Responsiveness to Diethylstilbestrol in Various Thyroidal States

By *J. Thomas Dowling, Norbert Freinkel and S. H. Ingbar.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

The frequent alterations of reproductive physiology in thyroidal disorders have prompted the suggestion that sensitivity to ovarian hormones may be influenced by metabolic status. To test this thesis in human subjects, the following studies were performed.

Eleven euthyroid, 4 thyrotoxic and 3 myxedematous women were given 30 mg. of diethylstilbestrol by mouth daily. The large doses were designed to minimize the variable contribution of endogenous estrogen elaboration. On alternate days during control and experimental periods, vaginal smears were obtained as an index of the genital response to estrogen. To assess systemic estrogen action, blood specimens were secured at similar intervals and assayed for serum precipitable iodine (SPI), and for the binding capacity of the specific thyroxine-binding plasma protein (TBP). In euthyroid subjects, full estrogenic effects upon vaginal epithelium were achieved after 5 to 17 days of treatment (mean: 10 days), whereas 18 to 29 days (mean: 21 days) were required in thyrotoxic and 6 days or less in myxedematous patients. The correlation coefficient between SPI and the temporal change in vaginal epithelium was +0.613 (p: 0.01). Systemic manifestations of estrogen action were similarly delayed in the hyperthyroid group. Thus, 21 to 28 days of diethylstilbestrol treatment were required for maximal augmentation of TBP-activity in thyrotoxic patients, whereas comparable changes occurred within 12 days in the sera of eumetabolic subjects.

Whether such differences in responses in thyroidal disorders are determined by variations in (a) rates of estrogen catabolism, (b) intrinsic tissue responsiveness, (c) availability of substrates and accessory factors, or (d) rates of cellular and plasma protein turnover, remains to be elucidated. However, from the data it may be concluded that the manifest response to constant quantities of estrogen is conditioned by metabolic status and is inversely proportional to general oxidative metabolism.

Hereditary Hyperparathyroidism Associated with Recurrent Pancreatitis

By *Charles E. Jackson.* Caylor-Nickel Clinic, Bluffton, Indiana.

The purpose of this paper is to present 5 and possibly 7 cases of hyperparathyroidism in two generations of one family associated with recurrent pancreatitis in at least two instances. During studies for pancreatitis in the mother of two young men who had had many episodes of pancreatitis, a serum calcium unexpectedly was found to be elevated to 12 mg. %. Subsequently, serum calciums of the brothers were found to be elevated (13 mg. %) and solitary parathyroid adenomas removed. Although no causal relationship has been postulated in the few other reports of pancreatitis associated with parathyroid adenomas, it seems possible that pancreatitis may result from stone formation in the pancreatic ducts secondary to the hyperparathyroidism.

Studies of the six siblings of the mother revealed two definite and two other probable cases of hyperparathyroidism. One brother who had had a tumor of the jaw removed was found to have a calcium of 12.5 mg. % and, subsequently, had a single parathyroid adenoma excised. A sister who had had several jaw tumors was found to have a calcium of 12.7 mg. %. A brother who had had jaw tumors and who had a calcium reported as 8.7 mg. % with a phosphorus of 2.3 mg. % is being studied further. Another brother who had had a jaw tumor removed and who died suddenly at age 40 was found to have extensive nephrocalcinosis at autopsy most likely indicating hyperparathyroidism, although his parathyroid glands were not examined. No evidence of other endocrine tumors was found in the individuals of his family.

The pedigrees of this family and the five other families reported in the literature are compatible with an autosomal dominant inheritance of at least some parathyroid adenomas, suggesting that the families of all cases of hyperparathyroidism be studied for this condition.

Metabolic Effects of Nilevar in Osteoporosis: Comparison of Intramuscular and Oral Routes of Administration

By *Edward Kessler, Richard Flaherty, Robert Cassidy, William P. Nelson, III and Sol S. Nelson.* Medical Research Laboratory and the Medical Service, V.A. Hospital, and the Department of Medicine, Albany Medical College, Albany, New York.

Nilevar, a synthetic steroid with anabolic properties, bears the chemical name 17 alpha-ethyl-17-hydroxy-19-nor-4-androsten-3-one. Its metabolic effects were investigated, and the efficacy of intramuscular and oral administration were compared in four elderly males with osteoporosis.

A constant diet of 2000 calories was given daily. Analyses of the diet revealed contents of nitrogen, 14.2 Gm., calcium, 750 mg., phosphorus, 960 mg., potassium, 93 mEq., and sodium, 159 mEq. Collections of urine, feces, and food rejects were pooled in three day periods. Blood was drawn at the beginning of each period. All specimens were analyzed for nitrogen, calcium, phosphorus, potassium, and sodium. Balances were studied for 21 periods (63 days). Nilevar, 50 mg. I-M, was administered during periods 5 through 9, and the same dose was given orally during periods 14 through 18.

Nitrogen: Nilevar, I-M, produced 1.5 times more nitrogen retention than did the oral administration (average retention—2.3 and 1.4 Gm./day, respectively). Positive balances were achieved through a reduction in urinary nitrogen excretion.

Calcium: Mild retention following I-M administration resulted from a reduction in stool calcium content. No definite changes were observed during oral administration.

Phosphorus: A mild increase in phosphorus retention during I-M administration was the result of a decrease in urinary output. Inconsistent changes were observed during oral administration.

Potassium: Retention during I-M administration was in excess of that explainable by the degree of nitrogen retention. Oral administration had little effect.

Sodium: Retention during I-M administration appeared to result from decreased urine sodium output in two subjects and from greater intake without change in output in two subjects. During oral administration the pattern was inconsistent.

Moieties examined in the serum were unchanged.

The results suggest that in equivalent doses I-M injection produces greater effects than oral administration. This could be the result of hepatic inactivation of the drug when given orally.

An Attempt to Separate Varieties of Osteoporosis Utilizing Radiocalcium

By *Richard Goldsmith*. Radioisotope Laboratory, Veterans Administration Hospital, Cincinnati.

The syndrome characterized by generalized loss of bone minerals in association with failure of normal matrix formation and unassociated with obvious abnormalities of calcium metabolism is called osteoporosis. Since many physiologically unrelated diseases are said to cause this bone disease it seemed important to study in detail the calcium kinetics of various patients thought to have typical osteoporosis. Four patients, each of whom had what was considered to be classical, advanced osteoporosis were studied. The etiologic diagnoses in these subjects were (1) chronic diffuse rheumatoid arthritis with long-standing cortisone treatment; (2) bronchial asthma associated with prolonged bed rest and cortisone treatment; (3) long-standing hypogonadism; and (4) old age combined with inactivity and poor nutrition but without evidence of vitamin deficiency.

These patients were each studied utilizing a combination of calcium and phosphorus balance and radioactive calcium tracer technics. The first three patients (category 1, 2, and 3 above) showed available calcium pools of similar size. The fourth patient (category 4 above) showed a pool size approximately 10 times greater than that of any of the other patients. Furthermore, this subject demonstrated very little turnover of radiocalcium. The total excretion of radiocalcium during the first 7 days of study on the first 3 patients averaged 15% of the administered dose. That for the fourth patient during the same period was 4%. Studies were performed to see if the calcium content of the diet or if the presence of a negative or a positive calcium balance at the time of study could account for this difference. They could not. The pool size in each of the first three patients was less than that found in any of 3 patients studied with hyperparathyroidism. The pool size found in the fourth patient was larger. The data on the first three patients fit the classical concept of osteoporosis. The data on the fourth patient show evidence of more bone formation than normal. It seems likely from these data that there are different physiologic mechanisms that produce the disease of bone called osteoporosis.

Dialyzable and Non-dialyzable Components of Serum which Promote Sulfate Uptake by Cartilage from Hypophysectomized Rats in Vitro

By *William D. Salmon, Jr. and William H. Daughaday*. St. Louis, Missouri.

Serum from normal rats or from hypophysectomized rats treated with growth hormone stimulates greatly the sulfate uptake in vitro of cartilage from hypophysectomized rats. Because Bostrom, Roden, and Vestermark have found that glutamine stimulates sulfate incorporation by beef cartilage in vitro, the effects of amino acids on sulfate uptake by rat cartilage during incubation in vitro for 24 hours have been tested.

Glutamine, added to a buffer medium, failed to stimulate sulfate uptake by cartilage from either normal or hypophysectomized rats. Furthermore, glutamine did not augment the effect of dialyzed serum from normal rats.

The addition of 13 amino acids to the incubation medium (shown by Eagle to be essential for growth in vitro of HeLa cells) more than doubled the sulfate uptake of cartilage from hypophysectomized rats. When tested in groups, according to chemical structure, only the aliphatic, monoamino-monocarboxylic acids (leucine, isoleucine, threonine, and valine) stimulated sulfate incorporation, but the effect was only one-third that observed with the total amino acid mixture. None of these 4 amino acids individually had an effect equal to their combined effect. The addition of 9 vitamins to the incubation medium did not increase sulfate uptake by cartilage from hypophysectomized rats.

Only part of the sulfation-promoting activity of serum from normal rats was found dialyzable. Activity was recovered in the dialysate, and full activity was obtained when dialyzable and non-dialyzable components were recombined. Serum from hypophysectomized rats contained only the dialyzable sulfation-stimulating material. A non-dialyzable sulfation-promoting factor has been found in normal human serum but was not found in one pool of serum from 3 patients following complete surgical hypophysectomy.

Conclusion: The dialyzable sulfation-promoting

component of serum may be a mixture of L-amino acids, and not glutamine alone. The hormonally determined sulfation-stimulating factor is probably a protein.

A New Diagnostic Test For Early Diabetes Mellitus

By *Robert H. Unger and Leonard L. Madison.* Department of Internal Medicine, University of Texas Southwestern Medical School, and the Veterans Administration Hospital, Dallas.

Intravenously administered sodium tolbutamide produces a prompt and rapid fall in blood sugar in normal subjects, whereas mild diabetics exhibit a gradual decline. The purpose of this study was to determine if these differences might serve as a diagnostic test for early diabetes.

One gram of sodium tolbutamide was administered intravenously over a two-minute period and the blood sugar concentrations were measured at 20-minute intervals for the ensuing two hours. The responses of 15 mild diabetics with fasting blood sugars ranging from 72 to 187 mg. % were compared with those of 17 nondiabetics. The standard three-day preparatory diet preceded each test.

Striking difference in response was observed. Without exception the blood sugar levels of the diabetics declined slowly over two hours or more, while the normals exhibited precipitous falls in blood sugar within the first hour, followed by a return toward normal. Complete separation of the two groups was noted at 40 minutes after the injection; at that time the mean fall in blood sugar among the diabetics was 10.5% of the fasting level (S.D. 6.3), while the decline among the normal subjects averaged 45% of the fasting level (S.D. 11.7).

Although justifiable on empirical grounds alone, the rationale of the test is based on evidence, not yet incontrovertible, that sulfonylurea-induced hypoglycemia results from enhanced insulin release. The use of a non-glucose "betacytotropin" to test beta-cell function has theoretical advantages over both oral and intravenous glucose tolerance tests, permitting an evaluation of glucose disappearance unobscured by the caprices of gastrointestinal absorption of glucose which beset the oral glucose tolerance test and free of mass action effects induced by rapid intravenous administration of glucose.

Studies With a New Procedure For The Measurement of Insulin Sensitivity

By *N. Heller, N. Kalant, C. Gomberg and M. M. Hoffman.* Research Laboratory, Jewish General Hospital and the Departments of Medicine and Investigative Medicine, McGill University, Montreal.

Most commonly used methods for the measurement of insulin sensitivity are performed at normal blood sugar levels. In these circumstances, hypoglycemia induced by the test dose of insulin acts as a stimulus to homeostatic mechanisms which then mask the full response to insulin. To avoid this difficulty, a procedure has been established to permit the measurement of glucose utilization and insulin sensitivity in a single procedure, during moderate hyperglycemia.

The blood sugar is raised to 250–300 mg./100 ml. by an injection of glucose and maintained at this level by a glucose infusion. The infusion is stopped and the half-life of blood glucose (t ½) determined by frequent sampling over the next 30 minutes. Insulin is then administered intravenously and the blood sugar half-life remeasured over the next 30 minutes. The difference between the two measurements (Δt ½) is an index of insulin effect; if no insulin is given, the two measurements are identical.

The t ½ in fifty normal subjects ranged from 20–85 minutes. As the t ½ rose within this range there was an equal rise in the Δ t ½ so that insulin reduced the half-life to 20 $\pm$ 1 minutes. There was no difference in effect between insulin doses of 0.03 μ./Kg. and 0.1 μ./Kg. It appears that the rate of glucose utilization was limited at this level by a factor other than insulin.

A group of middle-aged "stable" diabetics was studied three days after discontinuing insulin therapy. The range of t ½ was 75–275 minutes. As the t ½ rose there was an equal rise in Δ t ½, insulin reducing the half-life to 104 $\pm$ 10 minutes. For a given value of t ½, insulin had a greater effect in normals than in the diabetics. This might have been the result of an increase in the non-insulin limiting factor or of a decrease in the effectiveness of the administered insulin. All diabetic subjects demonstrated increased retention of insulin I^{131} in the plasma, and "binding" of insulin to γ globulin.

Studies were made on a second group of middle-aged diabetics, previously untreated with insulin but having the same range of ante-cibal blood sugar as the first group after discontinuing therapy. The t ½ ranged from 76–120 minutes. Insulin had an effect intermediate between those of normals and treated diabetics, reducing the half-life to 47 $\pm$ 10 minutes. These patients had normal retention of insulin I^{131} in the plasma and no binding to γ globulin. The differences between the three groups are compatible with the concept that insulin treatment induces the development of antibodies which cause retention of the insulin in the plasma and thus reduce its effectiveness.

Variability in Absorption of Insulin I^{131} After Subcutaneous and Intramuscular Injection

By *Edward W. Moore, Marvin L. Mitchell and Thomas C. Chalmers.* Lemuel Shattuck Hospital, Boston.

Variability in the rate of insulin absorption from the tissues was studied as a possible explanation for

some of the difficulties encountered in regulating "brittle" diabetes.

Insulin I^{131} (0.02 mg., 15–30 μc.) was administered to 11 normal and 12 diabetic subjects (insulin requirement 15–70 units). Each subject received simultaneous injections in the triceps area of each arm. Radioactivity in the injection site was measured for eight hours and plotted as percentage of initial count.

During the first two to three hours, the insulin I^{131} disappeared exponentially, but with a marked variation in rates of disappearance. Mean half-time from 15 subcutaneous injections in normal subjects was 174 ± 57 (1 S.D.) minutes. The diabetic group was similar (154 ± 68; N = 27), except for two patients. In one of these the half-time was 23½ hours, and in the other 16 hours on two occasions. In each instance, however, a normal rate of disappearance was observed simultaneously in the contralateral arm, suggesting that these extremely prolonged disappearance rates were due to injection into relatively avascular tissues.

Studies designed to elucidate the cause of this variability revealed less variation in disappearance rates following intramuscular injections, with similar half-times in normals and diabetics ($P < 0.3$). Intramuscular absorption was only slightly faster than subcutaneous when compared by simultaneous injections in 7 normal and 7 diabetic subjects ($P < 0.10$).

Dilution of the administered tracer dose with phosphate buffer (0.25–1.0 cc., pH 7.39) shortened the mean half-times slightly in diabetics and normals, while dilution with 10–20 units of carrier insulin prolonged absorption somewhat in five diabetics ($P < 0.10$).

These data suggest that there is considerable variability in rate of insulin absorption from subcutaneous tissues which is not dependent on volume or concentration of insulin injected. This variability may be partly responsible for difficulties encountered in regulating some diabetic patients.

The Metabolism of D-Ribose in Man

By *Stanton Segal and Joseph Foley.* National Institutes of Health, Metabolic Diseases Branch, Department of Health, Education, and Welfare, Bethesda, Maryland.

The phosphorylated derivative of ribose, ribose 5-phosphate, has been demonstrated to be a key intermediate in the oxidative or pentose phosphate pathway of glucose metabolism in animals and plants. However, little is known about ribose metabolism in man. In order to elucidate the physiologic disposition and metabolic fate of this important pentose, studies in man have been performed using unlabeled and C^{14} ribose.

Three to 20 Gm. of ribose were administered intravenously to normal subjects by single injection and constant infusion. Blood ribose, glucose, inorganic phosphate and pyruvate levels were determined. After the rapid (15 min.) injection of amounts over 3 Gm. a lag was noted before onset of an exponential disappearance of the ribose from blood. The rate constant of disappearance varied inversely with the dose. Intravenous insulin (0.1 U/Kg.) accelerated the ribose disappearance from blood. This effect of insulin on ribose was independent of insulin-induced hypoglycemia.

Blood glucose levels were depressed to 35 to 60% of control values by single injection of 10 or 20 Gm. of ribose or by constant infusion of smaller quantities. Blood inorganic phosphate and pyruvate levels were only slightly altered.

When 5 μc of ribose-1-C^{14} was added to a 20 Gm. infusion, the C^{14} activity in the blood decreased exponentially but with a smaller rate constant than chemically determined ribose. With time an increasing ratio of radioactivity to ribose was observed in urine. These two findings suggest the presence of a ribose metabolite in blood and urine. Twenty-four % of retained C^{14} was excreted as $C^{14}O_2$ in 6 hours. In contrast, when a 5 μc tracer dose (2.5 mg.) was given, $C^{14}O_2$ appeared at a faster rate and 49% was recovered in $C^{14}O_2$ in 6 hours.

The results demonstrate that ribose is metabolized by man, that it is an insulin responsive sugar and that its administration significantly alters glucose metabolism in normal man.

The Effect of Cortisone on the Response to Vitamin D

By *Marc Moldawer and Philip H. Henneman,* Boston.

In previous studies we interpreted increased calcium absorption of patients with sarcoidosis and hypercalcemia as suggesting the presence of excessive vitamin D or D-like substances, or hypersensitivity to vitamin D. Correction by cortisone of the altered calcium metabolism in such patients, and other observations, have led to the concept of an "anti-vitamin D action" of cortisone. This concept is not supported by the following direct observations.

A complete balance study was performed on a patient with rheumatoid arthritis and metastatic calcification three months after excessive vitamin D had been discontinued. Increased calcium absorption and hypercalcuria, indicative of persistent vitamin D effect, were unaltered by cortisone, while hypercalcemia was minimally decreased. A second patient with rheumatoid arthritis and metastatic calcification underwent complete balance study five months after discontinuing excessive vitamin D therapy. Cortisone decreased the hypercalcemia, but did not alter the hypercalcuria or the normal calcium absorption. Cortisone administration did not significantly alter calcium absorption during

balance studies on seven additional patients, nor did ACTH in eight other patients. Calcium absorption has been found to be normal in nine patients with Cushing's syndrome.

A balance study was carried out in a patient with pseudohypoparathyroidism, who was chosen because changes in parathyroid activity could not mask cortisone or vitamin D effects. This patient showed increased calcium absorption, hypercalcuria, and mild hypercalcemia due to daily administration throughout the study of 300,000 units of vitamin D. Cortisone produced typical nitrogen loss, but had no effect on serum, urinary, or fecal calcium levels.

These observations suggest that neither cortisone nor endogenous corticosteroids affect calcium absorption due to endogenous or dietary vitamin D. It is concluded that cortisone does not antagonize the response to vitamin D.

The Effect of Pitressin on the Production of Plasma Corticoids by the Adrenal Cortex

By *D. M. Bergenstal, W. W. Tullner, H. J. Levine and S. J. Jackson.* Endocrinology Branch, National Cancer Institute, Bethesda, Maryland.

The control of ACTH release by the pituitary is intimately related to certain parts of the central nervous system, including the posterior lobe of the pituitary. The intravenous administration of 1 to 2 units of Pitressin over a two minute period has produced in two patients a twofold increase in plasma Porter-Silber reacting corticoids within 60 minutes. Saline control test shows no significant change in steroid levels. Following hypophysectomy in one of these patients the Pitressin test was negative, and when the patient's adrenals were stimulated with intravenous ACTH and Pitressin given during the infusion, no acute increase in corticoid output was observed. Two post-hypophysectomy patients and one patient with pituitary adenoma failed to give a significant response to the Pitressin test. Studies were also performed in the intact and hypophysectomized dog using a technic in which the adrenal vein is catheterized and direct adrenal venous blood can be collected for analysis. In an intact dog 2 to 4 units of Pitressin intravenously gave an increase from 40γ% to 95γ% in corticosteroids while one unit of ACTH gave a smaller response. In a hypophysectomized dog 2 and 4 units of Pitressin produced similar rises in corticosteroids from control levels of 5γ% to 15γ% while 10 milliunits of ACTH gave levels of 55γ%. Similar results have been observed in additional hypophysectomized animals. The markedly greater response observed in the hypophysectomized dog as compared to the intact dog during ACTH administration is in contradistinction to that seen when Pitressin is given, and would indicate that Pitressin stimulates marked corticosteroid production in the presence of an intact pituitary and to a less degree in the hypophysectomized animal. Whether the Pitressin contains some specific substance other than antidiuretic hormone which is responsible for this effect is being studied, using purified vasopressin and oxytocin.

Adrenocrotical Activity During Labor in Normal Pregnancy

By *N. S. Assali and E. McKay.* Department of Obstetrics and Gynecology, University of California, Los Angeles.

It has been previously demonstrated that the plasma levels of 17-hydroxy-corticosteroids (17-OHCS) are strikingly elevated during the latter part of pregnancy (Assali, et al.) and that these high levels may be due to submaximally stimulated adrenal cortex of the mother by endogenous ACTH (Garst and Assali). The present study was undertaken in order to investigate (a) whether labor in normal pregnancy constitutes an added stress to the maternal organism and (b) whether the maternal adrenals which are already submaximally stimulated are capable of responding to such an additional stress.

Twenty subjects with normal pregnancy were studied. Control plasma determinations of 17-OHCS were made just before or at the onset of labor and were repeated at frequent intervals during the first and second stage of labor, at the time of delivery and again 2 to 6 days postpartum.

The results show that in each case the control plasma levels of 17-OHCS were much higher than those of nonpregnant subjects but were of the same magnitude of the 2 series of pregnant patients reported previously. However, despite the elevated control levels, labor produced a further increase in the levels of 17-OHCS. The increase was progressive and reached its maximum during the second stage of labor. Following delivery, plasma levels fell to nearly control values.

It is concluded that (1) labor constitutes a stress to the maternal organism which becomes more severe as labor progresses; (2) the maternal adrenal cortex is still capable of responding to added stressful stimuli despite the fact that it is already working in a somewhat high gear; and (3) the available evidence tends to indicate that sources other than the maternal adrenal cortex are contributing to the high glucocorticoids in pregnancy.

Plasma Corticosterone and Hydrocortisone Levels in Man

By *Ralph E. Peterson.* National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

Through the development of a specific isotope dilution assay, it has been possible to measure

plasma concentrations of corticosterone in man. Thirty normal subjects were found to have levels of 0.4 to 2.0 μg. %, mean 1.1 ± 0.4. The ratio of plasma hydrocortisone to corticosterone in normal subjects was found to average 13:1. Adrenal disorders associated with a decreased production of hydrocortisone (Addison's disease, hypopituitarism, and the adrenogenital syndrome) were found to have low or unmeasurable levels of corticosterone. In Cushing's syndrome, increased plasma hydrocortisone levels were not always associated with significant elevations in plasma corticosterone. Experimentally induced alterations in adrenocortical function were associated with changes in plasma levels of both corticosterone and hydrocortisone. Intravenous ACTH administered continuously for 8 to 72 hours decreased the plasma hydrocortisone-to-corticosterone ratio to about 4:1; however, during the 8 to 72 hour period, the ratio remained constant. A diurnal variation in the corticosterone level was found to closely parallel the hydrocortisone level.

Aldosterone Excretion in Late Normal Pregnancy

By *Jacques Genest, Wojciech Nowaczynski, Erich Koiw, Jean-Marc Pépin, Bernard Thérien and Barna Vityé.* Clinical Research Department, Hôtel-Dieu Hospital, Montreal.

Determinations of urinary aldosterone in the last trimester of normal pregnancy were done with a new chemical method which permits the isolation of aldosterone in a very high degree of purity.

Ten 24 to 48 hour urinary aliquots were collected from six normal pregnant women in their 7th to 9th month of pregnancy. They were adjusted to pH 1 and extracted continuously with chloroform for 30 hours. Isolation of aldosterone from the crude neutral extract of urine was done by the successive use of 1° Silica gel column chromatography, 2° paper chromatographic separations on (a) propylene glycol/toluene system or preferably, ethylene glycol/toluene system, (b) isooctane/tertiary butanol-water system (Eberlein-Bongiovanni's E_2B), (c) benzene/55% aqueous methanol (Bush B_8 system). Aldosterone obtained in the last paper chromatographic separation was determined and identified by ultraviolet absorption, blue tetrazolium reaction, absorption spectra in 100% phosphoric acid and in concentrated sulfuric acid. The following results were obtained: 105, 108, 69, 59, 57, 20, 186, 66, 101 and 48 μg./day.

The aldosterone values determined by ultraviolet absorption at 239 mμ and blue tetrazolium reaction agreed within 5%, with the exception of one case. The chromogen spectra in 100% phosphoric acid and in concentrated sulfuric acid were identical to those of crystalline aldosterone. The aldosterone content in a pool of 268 L. of urine from normal women in their last trimester of pregnancy was 57.5 μg./L.

Normal levels of urinary aldosterone by our method vary between 2.2 and 10 μg./day with a mean of 5.1 μg.

Primary Hyperaldosteronism, Long-standing Potassium Depletion, and Pyelonephritis

By *Robert C. Muehrcke and Malcolm D. Milne.* Presbyterian Hospital, Chicago, and the Postgraduate Medical School of London, London.

Two women with primary hyperaldosteronism were studied during three metabolic balance periods: before and after potassium replacement and after removal of an aldosterone-secreting adenoma. They differed widely with regard to mode of presentation and clinical state. Patient #2 was asymptomatic and had no histologic abnormalities of the kidneys.

Both had hypertension; hypokalemic alkalosis; urinary potassium, calcium, and magnesium loss; sodium retention; and renal impairment.

The patients were deficient 1000 mEq. and 400 mEq. potassium, respectively. When 200 mEq. potassium chloride was given per day, the serum potassium level approached normal; the serum alkalosis subsided; the urinary excretion of alpha ketoglutaric acid and citric acid increased; the isosthenuria persisted; and the neutral or faintly alkaline urine—previously described in primary hyperaldosteronism—was found to be the result of potassium deficiency rather than the specific effect of the aldosterone-secreting tumor. Analyses of muscle indicated a moderate reduction of potassium and an elevation of sodium. Several months after adrenalectomy, the blood pressure of both patients had returned to preoperative levels.

In patient #1 surgical renal biopsy studies revealed chronic pyelonephritis. Review of kidney sections from other patients with *long-standing* primary hyperaldosteronism and other types of chronic potassium deficiency (e.g., Fanconi syndrome, diarrhea, etc.) were made. In all, histologic evidence of chronic pyelonephritis was found. Preliminary studies of potassium depleted rats indicate that they are particularly susceptible to pyelonephritis. The intimate relationship of potassium depletion to infection of the kidney is under study.

The Effect of Hydrocortisone on Simultaneously Determined Albumin Turnover and Nitrogen Balance

By *W. B. Blythe, F. L. Iber, I. Werner, M. E. Rubini, P. G. Frick and W. H. Meroney.* Department of Metabolism, Walter Reed Army Institute of Research, Washington, D. C.

Negative nitrogen balance may be induced in the intact animal by certain adrenal steroids. The

mechanisms through which this is accomplished have not been elucidated; whether protein synthesis is inhibited or protein degradation accelerated is controversial. An experiment was devised in an attempt to differentiate between these two possibilities in man by measuring the effect of hydrocortisone on simultaneously determined albumin turnover and nitrogen balance.

Normal subjects, previously allowed to become equilibrated on a constant nitrogen, low sodium diet, were given human iodinated serum albumin (HISA) and were studied for approximately four weeks. Serial plasma specific activity, plasma albumin concentration, cumulative urinary excretion of I^{131}, and nitrogen balance were measured.

Half way through each turnover study, hydrocortisone, 300 mg./day, was given for seven days.

The amount of I^{131} remaining within the body was expressed as per cent of the amount given and plotted logarithmically against time. The curve obtained steepened significantly in each case during the period of hydrocortisone administration, indicating accelerated albumin degradation. The average one-half turnover times for the control and experimental periods were 12.5 days and 8.6 days. There was a significant increase in nitrogen excretion in all subjects when hydrocortisone was administered.

The amount of nitrogen which could have been derived from the increased degradation of albumin during the period of hydrocortisone administration ranged from 5–9.5 Gm. This comprises only about 20% of the increase in cumulative nitrogen loss during the same period.

It is concluded, therefore, that the breakdown rate of other proteins, in addition to albumin, is accelerated. The possibility that the synthesis of other proteins is inhibited, although the catabolism is increased, cannot be refuted by these data.

The Role of Nonhormonal Factors in the Impaired Water Diuresis Associated With Addison's Disease and Anterior Pituitary Insufficiency

By *Charles R. Kleeman and Morton H. Maxwell.* Department of Medicine, University of California Medical Center and the Veterans Administration Center, Los Angeles. (Supported by a grant from the Upjohn Company.)

Although the impaired water diuresis of adrenal and pituitary insufficiency generally responds to hydrocortisone-like steroids, its etiology is unclear. Evidence for a specific role of "excess" antidiuretic hormone is very inconclusive. The present studies attempt to evaluate the contribution of reduced glomerular filtration and "altered" proximal and distal tubular reabsorption of solutes on the impaired diuresis. Aminophylline was used to increase G.F.R. (Creatinine clearance) and to alter reabsorption of solute.

Sustained 1000 cc. positive water loads were maintained for seven hours in 3 Addisonian, and 5 anterior pituitary insufficiency patients off compound E or F; 3 normals, and 2 diabetes insipidus (D.I.) patients. When a maximal flow occurred, 0.5 Gm. of aminophylline was administered I.V. Its effects were compared with 200 mg. of I.V. hydrocortisone in these subjects. Water loads without drugs were evaluated in some cases. Urinary osmolality was measured by freezing point depression.

In spite of the water load all control urines in adrenal and pituitary deficient subjects were *hypertonic* (400–700 mOsm./L.). Maximal flows were 0.3–4.0 cc./min. Following aminophylline urinary flows *increased* 4 to 10 fold and all urines became *hypotonic* (100–200 milliosmols/L.). Creatinine clearance (G.F.R.) and solute excretion increased in all cases. Subsequent administration of hydrocortisone caused a qualitatively similar, but quantitatively greater diuresis (minimal Osm. 50–150 mOsm./L.). Changes in G.F.R. were comparable to those after aminophylline but solute excretion increased less.

In normals and D.I. subjects aminophylline increased G.F.R. slightly and total solute excretion 200 to 400 microOsm./min., but in every case urinary osmolality *increased.* Hydrocortisone had no effect.

Aminophylline and hydrocortisone did not block the effect of endogenous (hydropenia) or exogenous A.D.H. (I.V. Pitressin) in normal subjects.

Results suggest that aminophylline is capable of qualitatively reproducing the effects of hydrocortisone on water excretion in adrenal and pituitary deficient subjects, emphasizing the role of idiorenal factors in their impaired water diuresis.

The Metabolic Conversion in Man of 21-Carbon and 19-Carbon-1,4-Diene Steroids to 17-Ketosteroids

By *Maurice M. Pechet and Jane Claffey.* Massachusetts General Hospital and Harvard Medical School, Boston.

The metabolic actions of 21-carbon-diene steroids prednisone (Meti-E) and prednisolone (Meti-F) are qualitatively similar to those of cortisone and hydrocortisone except that sodium retention is less with meti-steroids. Further disparity lies in (1) limited conversion of administered 21-carbon-diene steroids to 17-ketosteroids; (2) limited conversion to 17-ketosteroids is principally by cleavage of the dihydroxy-acetone side chain prior to reduction of ring A, this sequence differing from that for 21-carbon natural steroids; (3) increased proportion of non-conjugated 21-carbon metabolites formed.

Complete metabolic balance studies were carried out on one normal, one panhypopituitary and three Addisonian female subjects with the following

16 steroids: (1) prednisone (Meti-E); (2) prednisolone; (Meti-F); (3) cortisone; (E); (4) hydrocortisone (F); (5) 20β-dihydro-prednisolone (Meti-U); (6) 17-desoxy-prednisolone (Meti-A); (7) 6-dehydro-prednisone (Triene-E); (8) 4-chloro-prednisone (4cl-Meti-E); (9) 14α-hydroxy-hydrocortisone; (10) Δ^4-androstene-3,17-dione; (11) 11βOH-Δ^4-androstene-3,17-dione; (12) 11βOH-$\Delta^{1,4}$-androstadiene-3,17-dione; (13) 1-dehydro-testosterone (Meti-testosterone); (14) 9αF,11βOH-$\Delta^{1,4}$-androstadiene-3,17-dione; (15) 9αF, 11βOH, 17α-methyl-testosterone; (16) 1-dehydro-17α-methyl-testosterone (methyl meti-testosterone).

Excretion as 21-carbon metabolites is largely in the unconjugated form (50–64%) for 21-carbon-1,4-diene steroids contrasted to 12–15% for cortisone. Conversion to 17-ketosteroids (Zimmermann reaction) is limited to 2–5% for 21-carbon-1,4-diene steroids contrasted to 10% for cortisone. This disparity is not explicable by differences only in the manner of cleavage of the dihydroxy-acetone side chain since 19-carbon-1,4-diene steroids are similarily limited in conversion to 17-ketosteroids: 0–5% for diene-steroids, 50–65% for natural 19-carbon steroids. In the metabolism of 21-carbon and 19-carbon natural steroids the oxidation-reduction reaction at C_{17} favors the 17-ketone, with predominate excretion of 17-ketosteroids over 17-alcoholic steroids. With 21-carbon and 19-carbon-1,4-diene steroids interconversion of 17-keto and 17-hydroxyl groups is unfavorable to 17-ketosteroid formation. This allows a study of pituitary-adrenal function by measurements of 17-ketosteroids utilizing suppression of the pituitary with small amounts of diene steroid Meti-E or Meti-F.

Observations on the Mineral Content of Bone in Derangements of Sodium Metabolism in Man

By *J. W. Agna and H. C. Knowles, Jr.* Metabolism Laboratory, Department of Internal Medicine, Cincinnati General Hospital, Cincinnati.

There is evidence that the bone Na concentration may decrease in acute Na depletion in experimental animals. Accordingly, observations have been made on the composition of rib, skull, and ilium obtained at autopsy in 16 normal subjects (sudden death) and in 14 patients with abnormal Na metabolism. Determinations were made of water, Ca, P, Na, K, Cl, and N. Cations were separated by column chromatography for flame photometric analysis. Values were expressed per Kg. of fat-free solids.

In the normals rib and ilium were similar in composition, while skull contained significantly greater concentrations of Na, Ca, and P and significantly lower concentrations of K and N. However, the concentration ratios of Na:Ca (mM:M) were constant and were 46.2, 47.4, and 46.5 for rib, ilium, and skull, respectively. The concentrations of K, Cl, and N were interrelated directly in linear fashion.

In the abnormal subjects significant changes were found in the concentrations of Na whether expressed per solids or per Ca content. Of 6 patients with decreased Na concentration ranging to 20%, 3 had metabolic acidosis, 2 had Na losing states, and one had marked dehydration with hypernatremia. Of 2 patients with Na concentration increase ranging to 20%, one had experienced salt loading because of hyponatremia, and one had cardiac failure. Of 6 patients with normal bone Na, 3 had cardiac failure and 3 acute renal failure. Alterations in Na were found most commonly in rib and least in ilium. No relation was apparent between the concentrations of Na in plasma and bone. No changes were noted in concentrations of the other determined substances.

The observations give evidence that the concentration of bone Na in man may be altered in derangements of Na metabolism.

Simple and Accurate Simultaneous Measurement of Exchangeable Sodium and Potassium in Man

By *Richard R. Paton.* National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

A simple and accurate method for simultaneous measurement of 24 hour exchangeable sodium and potassium with Na^{24} and K^{42} has been developed using only a flame photometer and a single counting method. Partial separation of Na^{24} and K^{42} is provided by body fluids having different sodium to potassium ratios. Serum and saliva (or urine) are especially adaptable, are easily collected and afford considerable separation—the serum ratio (mEq./L. sodium:mEq./L. potassium) being about 140:4 and the saliva ratio being about 20:20. Using 1.5 μc./Kg. of K^{42} and 0.33 μc./Kg. of Na^{24}, K^{42} will predominate in the saliva while Na^{24} will predominate in the serum. Under these circumstances simultaneous equations allow accurate determination of specific activities once the serum and saliva sodium and potassium concentrations are known.

Two to five ml. samples give satisfactory counting rates with either beta or gamma counting methods. When flame photometer and counting measurements are accurate to ±1%, the specific activities of sodium and potassium are accurate to ±2% and ±3%, respectively.

The 24 hour Na^{24} excretion can be calculated from the total sodium content of the 24-hour urine and the observation that the mean 24 hour urinary sodium specific activity is 106% (103%–110%) of the specific activity at equilibrium (24 hours). K^{42} excretion is the difference between total radioactivity excretion and Na^{24} excretion. This method of measuring isotope excretion introduces less than

0.5% additional error in the final values of 24 hour exchangeable sodium and potassium.

In six studies, values for 24 hour exchangeable sodium and potassium by the biologic separation method described above are in close agreement with measurements done simultaneously using differential counting methods, and with values in the literature.

The Effects of Alkaline Potassium Salts on Plasma Bicarbonate and pH

By *Blake Cady, Charles K. McSherry and Kathleen E. Roberts.* Department of Medicine, Memorial Center, Andre and Bella Meyer Physiology Laboratories, Sloan-Kettering Institute, and the Cornell University College of Medicine, New York.

It has been stated that alkaline salts of potassium which are ingested in the normal diet are a source of plasma bicarbonate. Conversely, it has been postulated that the maintenance or elevation of extracellular bicarbonate requires the acquisition of sodium ions despite the fact that sodium is not ingested as an alkaline salt normally, but as sodium chloride. If the latter is true, it would therefore seem illogical that potassium salts are a source of extracellular bicarbonate.

The experiments reported here were carried out to determine the effects of alkaline potassium salts on plasma pH and bicarbonate levels. In these experiments dogs were infused with 160–200 mEq. of potassium acetate, glutamate or bicarbonate over a 5 to 7 hour period. (Potassium citrate was found to be too toxic and could not be used in acute experiments.)

The results show that in 12 out of 13 experiments the potassium salts produced no simultaneous elevation in plasma pH and bicarbonate. In only one experiment was an actual metabolic alkalosis produced, and this was mild. In one-third of the experiments there was actually a decrease in bicarbonate following the administration of the alkaline potassium solutions. These studies furnish additional evidence that under normal circumstances the extracellular bicarbonate is produced by the kidney and possibly other endogenous metabolic means and not by dietary alkaline potassium salts.

Failure of Change in Extracellular Volume to Alter Plasma Potassium Concentration

By *James M. Burnell, Henry Kleinberg and Belding H. Scribner.* Veterans Administration Hospital, Seattle, and the Department of Medicine, University of Washington, Seattle.

If the plasma potassium concentration proves to be a valid index of intracellular stores and therefore an accurate guide to potassium need, the plasma potassium concentration should not be affected by changes in the size of the extracellular space. Yet numerous observers have ascribed changes in the plasma potassium concentration to concomitant changes in the size of the extracellular space. The present study was undertaken to evaluate the importance of changes in the size of the extracellular space as a factor influencing the plasma potassium concentration.

Isotonic solutions of sodium salts were infused within two hours into six nephrectomized dogs. The infusion was calculated to increase the extracellular space by about 50% without changing its ionic concentration, particularly pH. Changes in the plasma potassium concentration were measured before, during and after the infusion, and corrected for nitrogen catabolism and pH effect.

In the six experiments there was an average increase of 53% in the size of the extracellular space as measured by infusion volume and checked by chloride space calculations. This resulted in an average decrease of 0.2 mEq./L. in the plasma potassium concentration. This was not considered significant, $p = 0.32$. Had simple dilution occurred the plasma potassium would have fallen 1.7 mEq./L. It was presumed that 0.50% to 1.50% of intracellular potassium transferred from the cells to maintain the plasma and extracellular potassium concentration.

Only relatively small amounts of potassium must move in and out of cells to maintain the plasma level constant even after marked alterations in extracellular volume. It therefore seems likely that in patients the serum potassium concentration is virtually independent of the size of the extracellular space.

Influence of Potassium Deficiency on Response to an Acidifying Salt

By *M. E. Rubini, W. B. Blythe, E. G. Herndon and W. H. Meroney.* Department of Metabolism, Walter Reed Army Institute of Research, Washington, D. C.

Renal and extra-renal compensation for chronic chloride excess was compared in normal and potassium-depleted subjects. Deficits exceeding 300 mEq. potassium, with little or no alkalosis, were produced gradually in normal subjects by limiting daily intake to a diet containing 1 mEq. sodium, .08 mEq. potassium, 1 Gm. protein, and 40 calories/Kg. body weight. Large oral loads of ammonium chloride were given for five days during the second week, when potassium conservation was prominent but cumulative loss was minor, and subsequently, when some 15% of body potassium had been lost. The composition of extracellular fluid and urine was measured, and internal and external balances of sodium, potassium, chloride, and phosphate were examined.

An altered response to the acidifying salt was evident in the mildly depleted subject, and was exaggerated when the potassium deficit had increased. Ammonia production was greatly enhanced in the potassium-depleted subjects, and as compared with the controls, ammonium comprised 40% more of the total cation increment in the urine. However, the urine of the depleted subjects was less acid, and the increase in titratable acid was curtailed. When ammonium chloride was discontinued, the urinary pH rose, despite an increasing potassium deficit, and increased ammonia production persisted.

During acid loading, potassium conservation was maintained, and the augmented potassium excretion induced in the control studies was much less pronounced. Urinary sodium loss, however, was exaggerated. Relative phosphate retention occurred.

Exchange of intracellular potassium for hydrogen ion, seen in normal and sodium-depleted subjects given acidifying salts, was reduced in the potassium-depleted subjects. Some of the sodium retained during potassium depletion, apparently replacing part of the intracellular deficit of potassium, was preferentially exchanged for hydrogen ion, and excreted prior to renal compensation for the acid load.

Experimental Potassium Depletion in Human Subjects: Effects of Normal and High-Sodium Intakes and of Desoxycorticosterone

By *Edward J. Huth and Russell D. Squires.* Chemical Section, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia.

Potassium depletion was produced in 4 studies (3 normal subjects) with milk rendered electrolyte-poor by a cation-anion exchange resin and reconstituted with chloride and bicarbonate salts in mEq. ratios of 3:1. In 3 studies, sodium intake was 348 mEq./day; in 1 study, 130. During 5-day control periods, potassium intake was 108 mEq./day; during potassium depletion, the intake averaged less than 1 mEq./day. Protein nitrogen intakes ranged from 15.3 to 17.0 Gm./day.

During initial depletion periods (without desoxycorticosterone) of 7 to 10 days' duration, subjects on high intake of sodium had cumulative balances of potassium, sodium, chloride, and nitrogen of −203 to −365 mEq., +378 to +512 mEq., +206 to +311 mEq., and −2.6 to −27.8 Gm., respectively. The same respective balances for the subject on normal sodium intake were −170 mEq., +226 mEq., +100 mEq., and −10.5 Gm.

During the second depletion period lasting 5 days (DOCA 20 mg./day and high-sodium intake), the additional cumulative balances of potassium, sodium, chloride, and nitrogen were −149 to −253 mEq., +497 to +727 mEq., +129 to +362 mEq., and −0.7 to −12.0 Gm., respectively.

Only 1 subject (high-sodium intake) developed a metabolic alkalosis with the following final arterial blood values: CO_2 content 30.1 mM./L., pH 7.47, and buffer base 58.0 mEq./L. This subject differed from subjects *not* developing alkalosis by showing distinctly lower ratios of sodium retained to potassium lost, higher ratios of sodium retained to chloride retained, the highest urinary acid (NH_4 + titratable acidity—HCO_3) excretion in the control period, and the smallest increase in this moiety during potassium depletion.

Potassium Depletion Following Self-Induced Diarrhea and Vomiting, Treated by Prolonged Psychotherapy

By *Victor E. Pollak, Glenn W. Flagg, Robert C. Muehrcke and Robert M. Kark.* Department of Medicine, Presbyterian Hospital, Cook County Hospital, and the University of Illinois; Department of Psychiatry, University of Illinois, Chicago.

Self-induced disability is a diagnosis frequently overlooked by internists and psychiatrists. Two such patients were studied intensively; each was seriously ill, and presented with severe metabolic disturbances, most notably profound hypokalemia. One had suffered episodes of severe vomiting and diarrhea for 7 years; the second had had anorexia nervosa and complained of severe intermittent diarrhea for 5 years. Both had been in hospital many times before the correct diagnosis was made.

The diagnosis of self-induced disability was suggested by their personality characteristics and abnormal social and family backgrounds, as well as by the absence of organic pathology. It was proven by obtaining evidence of excessive purgation in both, and by observing self-induced vomiting in one. In each a clear relationship between episodes of emotional insecurity and severe metabolic disturbances was demonstrated on many occasions. In each the family attitudes to the patient, to eating, and to cleansing of the bowel, resulted in a severe emotional conflict, with self-induced gastrointestinal disorders. There was little overt personality disturbance, but chronic latent personality disorders almost led to slow self-destruction. Both patients responded well to prolonged superficial psychotherapy by internists and psychiatrist.

In both, metabolic balance studies showed that potassium was lost mainly from the gastrointestinal tract. During periods of potassium deficiency there was inability to concentrate urine, and a decrease of clearances and phenolsulfonphthalein excretion; with potassium repletion these functions returned toward normal, but the ability to concentrate and to excrete a water load was still impaired 6 to 12 months later. Chronic pyelonephritis developed during the illness (serial renal biopsies) in both

patients; it has been reported in primary hyperaldosteronism and other causes of prolonged hypokalemia (Milne, Muehrcke, and Heard). Hydropic degeneration of the proximal convoluted tubules—previously reported in hypokalemia—was not observed even with potassium levels of 1.9 mg./L.

GASTROINTESTINAL SYSTEM

Non-inflammatory Parotid Enlargement—A Useful Physical Sign

By *John J. Duggan and Earle N. Rothbell.* V.A. Hospital, Syracuse, and the Department of Medicine, State University of New York, College of Medicine, Syracuse.

Non-inflammatory parotid enlargement has been recognized widely in areas of endemic malnutrition. Paradoxically, it also occurs when victims of kwashiorkor or starved prisoners of war are fed. It has been observed in European clinics in association with obesity, diabetes mellitus and liver disease. It has generally gone without note in our medical practice.

Fifty cases of parotid enlargement have been observed. Disturbed nutrition, usually due to alcoholism, provides the usual background. Specific deficiency states have not been recognized. Liver disease was present in 35 cases; obesity in 29; and disturbed glucose tolerance in 25 of the 42 cases in which complete observations were made. The liver disease is not severe and the arterial blood pressure tends to be sustained or even elevated. Three examples of rapid swelling of the parotids following abstention from habitual high alcohol intake are included.

Unity among the observed cases, here and abroad, is not established. However, the concept that they are related to protein lack has been presented by others on the basis of circumstantial evidence. Experimental parotid enlargement has been produced by manipulation of dietary protein. These cases are compatible with such a concept if it be extended to include relative protein deficiency—normal protein intake in the form of excessive total calories.

Detection of parotid enlargement has been a useful clue to the presence of previous dietary aberration—notably, concealed alcoholism. Such parotid enlargement is an indication for the study of glucose tolerance.

Simultaneous Multi-Electrode Recording of Gastric Potential

By *Henry Colcher, George M. Katz and Edmund N. Goodman.* (With the technical assistance of Carolyn L. Dangler and Jerome Meyer.) Departments of Surgery and Medicine, Columbia University College of Physicians and Surgeons, New York. (Aided by grants from N.I.H., Rothschild Research Gift, and James O. McCue Research Gift.)

Instrumentation and technics have been developed for simultaneous recording of electric potential from various areas of the stomach. Different types of electrodes have been either applied against or sewn into the stomach of anesthetized dogs (Nembutal). Measurements were made either differentially or with respect to various skin areas.

These simultaneous recordings were made with a 6 channel Offner amplifier and recorder modified by constructing preamplifiers designed to provide the necessary high input resistance, high in-phase signal rejection and low drift. Interchangeable preamplifiers for intragastric pressure measurements with Statham strain gages were built.

The most stable and reproducible recordings were obtained by using chloridized silver electrodes sewn into the stomach wall and applied to the skin of the femoral area with saline liquid junctions.

Reproducible patterns exhibiting 12 to 18 waves per minute have been recorded from the upper third of the lesser curvature and 3 to 4 waves per minute from the lower half of the stomach. The latter was correlated with visible motility.

Urecholine (5 and 10 mg. s/c) resulted in small potential changes out of proportion to the marked increase in motility. The basic rhythm was unaffected. For clinical studies, simultaneous recordings from 5 gastric areas are obtainable by means of small silver electrodes mounted on a balloon.

Chloride "Concentration Threshold" in Human Gastric Secretion

By *B. I. Hirschowitz.* Ann Arbor, Michigan

The most characteristic change from the basal to the stimulated state of gastric secretion in man is an increase in total chloride concentration ($[Cl^-]$). In the basal state $[Cl^-]$ is almost invariably under 130 mEq./L.; this was found in 100 gastric analyses in normals and in patients with gastric or duodenal ulceration and with gastric cancer. Whatever stimulation is applied, the $[Cl^-]$ rises to between 140 and 174 mEq./L. in normals and in patients with benign peptic ulceration. The intravenous administration of histamine (0.3 mg. base/hr.) was found to give a reproducible steady state of maximal gastric secretion, whereas other means of stimulating gastric secretion do not maintain the stimulation long enough to be sure of obtaining unmixed stimulated samples. In normals the transition from the basal steady state ($[Cl^-] < 130$ mEq./L.) to the stimulated state ($[Cl^-] > 130$ mEq./L.) was accompanied

by abrupt change in the rate of secretion from one plateau (68 ± 43 ml./hr., basal) to another (180 ± 88 ml./hr., stimulated). ($t = 11.4$, $p < .001$, $n = 271$).

In 15 unselected patients with gastric cancer under maximal histamine stimulation, the chloride concentration did not exceed 135 mEq./L., whereas in 16 unselected patients with benign gastric ulceration, the gastric juice Cl^- always exceeded 132 mEq./L. While there were significant differences between these two diseases in the mean rate of volume secretion, pepsin and free and total H^+ ion concentrations, in increasing order, there was enough overlap between the levels in benign and malignant ulcers to limit their usefulness for the purposes of differential diagnosis. The combined use of $[H^+]$ and $[Cl^-]$ measurements, however, allowed 100% differentiation in the 31 patients studied.

The range of $[Cl^-]$ from 130–135 mEq./L. is seen as a threshold for gastric secretion and is useful for distinguishing the normal stimulated state from the normal basal state and from the hyporeactor state of which gastric cancer is a major example.

The Physiologic and Clinical Limitations of Blood Acid Protease (Blood Pepsin)

By *H. M. Spiro, E. Friedman and I. J. Poliner.* Yale University School of Medicine, New Haven.

Although there is an extra-gastric contribution to blood acid protease activity, the major contribution at pH 2.0 arises from the stomach; at a more alkaline pH, however, extra gastric "cathepsin" becomes a prominent factor. The straight-line relationship at pH 2.0, in normal subjects, between blood acid protease and post-histamine gastric pepsin, together with the demonstration that histamine truly stimulates gastric pepsin, permits interpretation of blood acid protease levels to indicate the potential peptic secretory ability of the stomach. Some limitations in physiologic studies must be considered, however. Although the blood pepsin level is most closely related to post-histamine gastric pepsin, a constant relation is no longer maintained following acute changes in gastric pepsin. In addition, immediately after subtotal gastrectomy, or in diffuse superficial gastritis, pepsin is released into the blood to give high levels without an increase (and often a decrease or absence) of gastric pepsin. Only in the *chronic unstimulated* state does blood pepsin (and presumably therefore uropepsin) reflect the secretory potential and integrity of the gastric glands. In evaluating the effect of any drug on gastric secretion by the indirect route, the presence of renal disease or of inflammatory disease of the stomach wall and the effect of the drug on these factors must be evaluated.

The relation between blood pepsin and gastric acid levels is only incidental, depending on the general height of gastric secretion. High blood pepsin (or uropepsin) levels should not be taken to indicate hypersecretion of gastric acid. Pepsin, acid, and intrinsic factor function are the products of separate cells and therefore are secreted independently. In gastric secretory failure, acid usually disappears before pepsin, and pepsin before intrinsic factor. Because of this, the presence of pepsin in the blood indicates nothing certain about the presence of acid in the stomach. Very low levels of blood pepsin, however, suggest that parietal and chief cells have atrophied. Since pepsin usually fails before intrinsic factor, low blood pepsin levels do not necessarily mean that intrinsic factor function has disappeared. The converse, however, is true; high blood pepsin levels almost invariably predict a normal radioactive B-12 uptake.

Gastrodialysis with a Semi-Permeable Membrane Bag

By *Paul R. Schloerb.* Department of Surgery, University of Kansas School of Medicine, Kansas City, Kansas and V.A. Hospital, Kansas City, Missouri.

The feasibility of selective removal of water, electrolytes, NPN, and other crystalloids from the blood with an intra-intestinal cellophane tube, containing an appropriate dialysate solution, has been previously reported by this laboratory.

A collodion-glycerine semi-permeable membrane bag has been devised for use in the stomach in an attempt to accomplish the same result, and its application to clinical problems, including renal failure, is being developed.

Using bilaterally nephrectomized dogs, a normal human, and three uremic patients, in evaluation studies, gastric intubation has been accomplished, or the tube has been swallowed, and dialysate fluid, similar in electrolyte composition to gastric contents, is infused into the bag through a small plastic tube. Sucrose or glucose is added to remove water. The rates of exchange of H^+, Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, P, urea N, NPN, and water have been determined, and the selective rates of removal of water, potassium, phosphorus, urea N, and NPN have been measured.

With a bag of 300 $cm.^2$ surface area and 300 ml. volume, a serum BUN (and gastric urea N) of 130 mg.%, and a perfusion rate of 1200 ml./hr., 312 mg./hr. of urea nitrogen and 3.0 mEq./hr. of potassium were removed. No decrease in removal rate during several hours perfusion was observed.

Preliminary results from these continuing studies indicate that gastrodialysis with a collodion-glycerine bag may be a feasible method for the selective removal of potassium and toxic accumulated metabolites in acute renal failure, for the selective removal of water in hyperhydration states, and for the adjustment of electrolyte derangements.

Diurnal Variation of Uropepsinogen and Its Relation to Adrenal Function

By *J. A. Vennes, R. P. Doe, D. Gleason and E. B. Flink.* VA Hospital, and the Departments of Medicine and Pathology, University of Minnesota, Minneapolis.

Possible adrenal regulation of gastric function has received much attention recently. Urinary pepsinogen is a valid index of gastric peptic activity in the basal state. The rise in uropepsinogen after administration of glucocorticoids or ACTH may be due to end-organ action on the gastric mucosa with increased pepsinogen output, or on the kidney, resulting in augmented renal pepsinogen clearance.

Eight normal subjects were studied with plasma samples and urine collections every three hours, while on a constant diet. A marked diurnal variation in urinary excretion of pepsinogen was found, with a maximum three hour total of 101 mg. at 9 A.M., falling gradually to 40 mg. in the 3–6 A.M. period, then rising abruptly. There was a striking correlation between uropepsinogen and 17-hydroxycorticosteroid excretion, except that the minimum uropepsinogen excretion occurred about 3 hours later than that of corticoids. Creatinine clearance during this period varied about ±10%, and was poorly correlated with the changes in the other two modalities.

A group of subjects with proven adrenal cortical insufficiency was studied every 3 hours under identical conditions as the normal subjects, except that 5 mg. hydrocortisone was given intravenously every 3 hours. The diurnal variation of uropepsinogen excretion disappeared, as did that of corticoids. Creatinine clearance varied about as in the normal subjects.

It is concluded that there is a marked diurnal variation in uropepsinogen excretion and that this variation is dependent on a rhythmic secretion of the adrenal cortex. This variation is poorly correlated with creatinine clearance. The maximal effect of 17-hydroxycorticosteroids on uropepsinogen excretion appears to occur in about three hours. It appears that valid daily uropepsinogen excretion values require that the total 24 hour urine specimen be collected, in light of the above noted diurnal variation.

Hyperlipemia and Pancreatitis: in Man and in Experimental Animals

By *C. I. Wang, F. Paronetto and D. Adlersberg.* Department of Medicine, The Mount Sinai Hospital, New York.

The association of abdominal crises highly suggestive of pancreatitis and idiopathic hyperlipemia was observed in 8 patients. Their sera were milky in appearance in the "fasting" state. During the crises serum lipids were markedly elevated with average cholesterol level of 643, phospholipids of 768 and total lipids of 4270 mg.%. After institution of a regimen consisting of administration of intravenous fluids and of diminished or no food intake the sera became clear. Serum lipids decreased considerably: cholesterol 254, phospholipids 328 and total lipids 1146 mg.%.

A study on the relation of experimentally produced pancreatitis and serum lipids was performed in 35 rabbits and 11 dogs. Instillation of a potent staphylococcus toxin into the ligated pancreatic duct in these animals produced acute fulminating pancreatitis and concomitant lactescence of the serum; the latter lasted 1–4 days. In rabbits the maximum elevation of serum lipids was observed on the second day and averaged 109 for cholesterol, 286 for phospholipids and 1142 mg.% for total lipids; the corresponding values for normal rabbits were 50, 105 and 350 mg.%, respectively. Ligation and injection of saline into the pancreatic duct did not affect the serum lipids. In dogs, experimentally produced pancreatitis also caused elevation of serum lipids for 1–4 days but to a lesser degree. Serum cholesterol averaged 295, phospholipids 469 and total lipids 1126 mg.%; the corresponding figures for normal dogs were 170, 340 and 765 mg.%, respectively. In control experiments ligation and instillation of saline failed to produce elevation of serum lipids.

Thus a decided hyperlipemia may be produced in animals by experimental pancreatitis.

Serum Lipids and Lipid Enzymes in Acute Pancreatitis with Lipemia

By *Harold H. Orvis and John M. Evans.* Department of Medicine, The George Washington University School of Medicine, Washington, D. C.

A patient with pancreatitis presenting with marked lipemia has afforded us the opportunity to compare serum proteins, lipid fractions and enzymes in the same individual while in a grossly abnormal state and subsequently when the process had subsided.

The patient was a 38-year-old colored male with a history of alcoholism and the typical findings of acute pancreatitis. Serum amylase and lipase were elevated. Fat laden stools were passed during the early weeks of therapy which, for the first six days, consisted of intravenous fluids and parasympatholytic drugs.

On admission the serum total lipids were 11,000 mg.% of which 1100 mg.% were cholesterol. It was observed that total lipid, cholesterol and its esters and phospholid gradually declined to normal values over a six week period as did the serum lipase. On the other hand, beta lipoprotein cholesterol rose from 86% to 93.5% (of total) during the first 10 days of illness and then gradually declined to the low normal level of 71.2%, suggesting a shift in

lipid transport possibly related to but lagging behind the initial lipemia. This shift correlated with depression of serum albumin, determined by serial paper electrophoresis, which reached a minimum on the tenth day.

Blood cholesterase activity (Sperry) was depressed at the height of the illness when considered in terms of percent esterification. In absolute terms, however, esterification of 90 mg.% of free cholesterol in 24 hours indicated significant cholesterase activity.

In vitro assays of clearing factor revealed inhibition of clearing of the lipemic serum by the patient's own post-heparin serum as well as reduced clearing by post-heparin serum from a normal subject. That clearing factor was produced was indicated by typical changes in the electrophoretic pattern of the lipoproteins after heparin. These observations suggest the presence of an endogenous inhibitor of the patient's lipoprotein lipase.

On the basis of these findings, it is concluded that the lipemia observed in this patient was a result of mobilization of endogenous lipid, probably as a result of increased serum lipase. With sustained hypercholesterolemia, a shift in lipoprotein transport occurred as evidenced by an increase in beta lipoprotein cholesterol. Although lipoprotein lipase could be demonstrated in post-heparin serum, the presence of an inhibitor prevented frank clearing of lipemic serum. The reversion of all lipid fractions to normal is in contrast to previously reported cases.

Lipase Activation by Heparin and Phosphorylated Hesperidin

By *Harold H. Orvis and John M. Evans.* Department of Medicine, The George Washington University School of Medicine, Washington, D. C.

It has been demonstrated that clearing factor is a lipase, activated by heparin, that will catalyze the hydrolysis of triglycerides when the latter are associated with protein. Phosphorylated hesperidin has also been shown to clear lipemic serum, by in vitro as well as in vivo technics. However, the mechanism of action of hesperidin has not been defined. We have studied the lipase effect of hesperidin as compared to heparin and believe a common or similar enzyme system is responsible.

Paper electrophoresis of lipemic serum after heparin administration reveals increased mobility of lipoproteins. This is due to the increased fatty acid content of the serum resulting from the action of the heparin activated lipase. We have obtained identical changes in the lipoproteins of lipemic serum after hesperidin with doses of 200 to 600 mg. administered intravenously.

This action was further studied by comparing the lipase activity of the two compounds in the production of fatty acid from an olive oil emulsion incubated with post-injection serum of each. It was observed that after 20 hours, there was a greater increase in titratable acidity with the post-heparin serum. However, at 60 hours, the post-hesperidin serum produces equal or greater titratable acidity.

To test the hypothesis that the enzyme involved was the same for both compounds, in vitro clearing was studied after introducing compounds known to block the heparin lipase action. It was observed that sodium taurocholate, quinine and protamine similarly inhibited clearing of lipemic serum when incubated with post-heparin or post-hesperidin serum. One difference has been consistently demonstrated in the in vitro systems. Clearing with post-hesperidin serum is slower than with post-heparin serum, requiring about 6 hours to reach the end point achieved by the latter in one half hour.

On the basis of these results it is concluded that a similar, and possibly identical, enzyme is present in post-heparin and post-hesperidin serum, although hesperidin activated lipase clears lipemic serum and releases fatty acid at a slower rate.

Operative Intestinal Arteriography

By *Robert Schobinger von Schowingen, George E. Blackman and Ru Kan Lin.* Departments of Surgery and Diagnostic Radiology, Roswell Park Memorial Institute, Buffalo, New York.

By injecting contrast material directly into certain arteries it is possible to visualize the vascular pattern of large segments of the intestinal tract during surgery. No direct reference to this procedure, which has been given the name of operative intestinal arteriography, has been found in the literature.

The patient must be tested against idiosyncrasies to the contrast medium (50% Hypaque) and the local anesthetic agent (1% Procaine hydrochloride) to be used. This is best done prior to the induction of anesthesia. A large Potter-Bucky diaphragm is put on the operating table and covered with a layer of foam rubber. The patient is so positioned as to center the principal area to be visualized on the mid-portion of the film. The correct positioning is verified with a scout film.

The selected artery and its principal branches are isolated near their origin and a plastic tubing (BD No. 444 T) is introduced into the main trunk of the artery. Vascular spasm is minimized by introducing 2–3 cc. of Procaine hydrochloride into the artery prior to the injection of contrast medium. By individually and alternatively clamping the various branches of a given main artery it is possible to carry out *selective* operative intestinal arteriography. An average of 15 cc. of dye is usually necessary to adequately visualize a given vascular territory.

The normal angiographic pattern of the intestine appears as a fine vascular network of rather uniform distribution in contradistinction to neoplastic processes, which exhibit an increased localized

radiopacity due to collection of contrast medium within the lesion. In most instances it is possible to outline the lesion in its entirety, and certain centrally ulcerated neoplasms did demonstrate a collection of contrast medium only in their actively growing edges.

The potentialities of operative intestinal arteriography are great and may lead to the detection of unsuspected lesions, whether benign or malignant. The procedure is simple and innocuous and is rapidly executed. No immediate or late untoward effects were observed in any instance.

The Occurrence of Hypoproteinemia in Infantile Gastroenteritis

By *David B. Coursin, Charles H. Kurtz, and Virginia Brown.* St. Joseph's Hospital, Lancaster, Pennsylvania.

Infantile gastroenteritis with its exacerbations, debilitating effects, and death continues to be a major pediatric problem despite progress in antibiotics and fluid replacement therapy. The present study was carried out in 50 infants with diarrhea in an effort to find additional factors that might be of importance in controlling these problems. Ultramicro blood chemistries were performed on admission and at intervals during the illness: pH; CO_2; Cl; Na; K; Ca; P; BUN; hematocrit; albumin; globulin; alpha, beta, and gamma globulins. A standard accepted therapeutic regime was followed using intravenous fluids, antibiotics, and gradual resumption of selected dietary intake.

Clinical evaluation and laboratory studies were correlated to evaluate the degree of illness as to mild, moderate, and severe. Electrolyte changes were those customarily seen in the course of diarrhea. However, the most interesting observations were those of measurable depletion of plasma proteins which coincided with the severity of the disease and the patient's progress. The more severe and chronic the diarrhea state, the more marked were the losses of body proteins, as shown by the low levels of plasma proteins as well as the decrease in volume content of the fluid spaces of the body. Electrolyte replacement solutions improved the chemical and volume pictures, but further diluted the protein concentrations. It was therefore found expedient to use plasma and protein hydrolysates in an effort to overcome this situation. The recognition of these protein needs has provided a better understanding of the complications of gastroenteritis and has pointed up their requirements in therapy.

Resistance and Reflex Function of the Lower Esophageal Sphincter

By *Bertram Fleshler, Thomas R. Hendrix, Philip Kramer and Franz J. Ingelfinger.* Evans Memorial, Massachusetts Memorial Hospitals, and Department of Medicine, Boston University School of Medicine, Boston.

The presence of a high pressure zone in the distal esophagus ("vestibule"), as demonstrated by direct manometry, has stimulated renewed interest in the sphincteric function of this area. To delineate this function further, answers to the following questions have been sought:

1. In the absence of swallowing, can the vestibule resist hydrostatic pressure from above, and what is the magnitude of the resistance? In ten young, erect subjects, radiopaque liquid was layered through a tube placed in the distal esophagus. Intraesophageal pressures were recorded using two water-filled, open-tipped catheters. The distal tip was immediately above the vestibule and the other 13 cm. proximal to it. The mean maximum height of the column supported in the esophagus was 12 cm. and was independent of diaphragmatic motion. The average maximal pressure rise at the distal tip during layering was 8.6 mm./Hg. This resistance to hydrostatic pressure cannot be explained by the pressure gradient from stomach to esophagus since this averaged 3.4 mm./Hg at the end of expiration ($p < 0.01$).

2. In the absence of swallowing, can sphincter relation be produced by esophageal stimuli? Peristalsis was initiated by a fixed balloon inflated in the mid-esophagus, and intraesophageal pressures recorded at three distal sites. In the vestibular area the characteristic high resting pressures fell coincident with or immediately after a peristaltic contraction was initiated by the balloon. It thus appears that relaxation of the vestibule occurs reflexly before the arrival of the peristaltic wave and does not require the presence of a bolus.

The resistance to a hydrostatic force exhibited by the vestibular area, and its reflex relaxation in response to esophageal stimulation, provides further evidence for a physiologically significant sphincter in this area.

INFECTIOUS DISEASE

The Efficacy of Penicillin Prophylaxis of Group A Hemolytic Streptococcal Infections in Rheumatic Children: A Controlled Study

By *Joseph M. Miller, Samuel L. Stancer and Benedict F. Massell.* House of the Good Samaritan and Harvard Medical School, Boston.

The present report compares the incidence of group A hemolytic streptococcal infections in ambu-

latory children recently recovered from acute rheumatic fever and receiving penicillin prophylaxis with the incidence of such infections in their healthy siblings not receiving prophylaxis.

Over a three-year period, 114 rheumatic children were maintained on oral penicillin prophylaxis, 400,000 units daily, and observed regularly with 152 of their siblings. For 18 months of this period, a group of 47 rheumatic children were given monthly injections of 1.2 million units of benzathine penicillin G and also observed in parallel with 83 of their siblings. Throat cultures and antistreptolysin-O determinations were performed on all subjects at monthly clinic visits and at home visits prompted by acute respiratory symptoms. Streptococcal infections were defined by the presence of at least one positive culture or by a significant ASL-O rise. Clinical correlates ranged from the asymptomatic "carrier" state to classical streptococcal tonsillopharyngitis with an intermediate spectrum of milder respiratory symptoms.

Both forms of penicillin affected a significant reduction of streptococcal disease among the rheumatic children as compared to the siblings. Oral penicillin reduced the over-all infection rate by 72%, the symptomatic disease rate by 83%, the asymptomatic disease rate by 64% and the rate of infections with associated ASL-O rise by 48%. Comparable reductions affected by long-acting repository penicillin were 89, 94, 84 and 92%, respectively. The poorer performance of oral penicillin may be related in part to variations in patient cooperation in taking an oral drug. No toxic reactions to oral penicillin were encountered in this study, while the parenteral form of benzathine penicillin G produced two instances of urticaria and considerable degrees of local discomfort.

Two recurrences of rheumatic fever during 6,545 patient-weeks of observation in the oral group and none during 2,498 patient-weeks of observation in the intramuscular group seem to demonstrate the usefulness of both forms of prophylaxis in a rheumatic fever prevention program.

Subacute Bacterial Endocarditis: Treatment with Oral Phenoxymethyl Penicillin (Penicillin V)

By *Edward L. Quinn, James M. Colville, Frank Cox, Jr. and Joseph Truant.* Division of Infectious Diseases, Henry Ford Hospital, Detroit.

A high dosage regimen of oral penicillin V has been shown to be an adequate substitute for parenteral Procaine penicillin G in the treatment of selected cases of bacterial endocarditis.

Studies on serum concentration and urinary excretion with 1,200 mg. (2,000,000 unit) doses of oral penicillin V and G indicated that penicillin V was more efficiently absorbed from the gastrointestinal tract. A high dosage regimen of oral penicillin V (1,200 mg. every four hours) produced penicillin serum concentrations equivalent to 600,000 to 1,200,000 units of Procaine penicillin administered intramuscularly. No acute or chronic toxicity was noted in 14 normal adult subjects and 25 hospitalized patients who received oral penicillin V in doses up to 7,600 mg. daily for periods of from one to 42 days.

Fifteen cases of bacterial endocarditis were treated with oral penicillin V. (a) Two cases of bacterial endocarditis due to *Streptococcus viridans* were successfully treated with penicillin V (1,200 mg. every four hours for six weeks). (b) A two week treatment schedule of penicillin V, probenecid and streptomycin proved satisfactory in nine of ten additional cases of *Streptococcus viridans* endocarditis. (c) A third group of three cases of bacterial endocarditis due to various *other* organisms was treated with penicillin V, probenecid and streptomycin administered over a four to six week period. In two of three patients in this group satisfactory clinical and bacteriologic remission was achieved.

Successful Short-Term Therapy of Enterococcal and Staphylococcal Endocarditis with Ristocetin

By *Monroe J. Romansky and J. Robert Holmes.* George Washington University Hospital, and District of Columbia General Hospital, Washington, D. C.

Ristocetin, an antibiotic derived from the actinomycete, *Nocardia lurida*, is particularly efficacious in vitro against gram-positive microorganisms and the *Mycobacterium tuberculosis*. Its characteristics include bactericidal action, low toxicity, effectiveness against antibiotic-resistant microorganisms, ability to delay markedly the development of antibiotic-resistant microorganisms in vitro, and an apparent lack of cross-resistance with other antibiotics. Ristocetin is administered intravenously.

Three patients with enterococcal endocarditis were successfully treated with Ristocetin. Two of these had received vigorous multiple antibiotic therapy for 4 and 12 months respectively, prior to the administration of Ristocetin. The third received Ristocetin as his only medication. The fourth patient, a 29-year-old heroin addict with staphylococcal endocarditis, was treated successfully with Ristocetin after 4 months of vigorous therapy with other antibiotics.

These 4 patients received 1.5 to 9 Gm. of Ristocetin intravenously per day in divided doses. The periods of therapy ranged from 13 to 24 days.

A satisfactory clinical and bacteriologic remission has been observed in these patients, the longest follow-up being 12 months.

The Sensitivity of 200 Strains of Hemolytic Staphylococci to 10 Antibiotics

By *Robert G. Petersdorf, James A. Curtin and Ivan L. Bennett, Jr.* The Johns Hopkins University, Baltimore.

Employing a tube dilution technic the bacteriostatic and bactericidal potencies of 10 antibiotics were determined for 100 strains of hemolytic staphylococci isolated from patients with severe infections and 100 strains from nasal carriers hospitalized for unrelated illnesses. Novobiocin was consistently the most potent bacteriostatic agent, 98% of strains being *inhibited* by less than 5 mcg./ml. In contrast, only 20% of strains were *killed* at this concentration. In the same concentration, Erythromycin was bacteriostatic for only 60% of strains and was rarely bactericidal, emphasizing the recent increase in hospital staphylococci resistant to this agent. A newer drug, Oleandomycin, was found to possess no advantage over Erythromycin. Although only 30% of staphylococci were inhibited by 5 units of penicillin/ml., 60% failed to grow at 50 units, a level readily obtained in vivo by intravenous administration of large doses coupled with Benemid. In addition, penicillin was bactericidal more frequently than Novobiocin or Erythromycin, an important advantage. Neomycin and Bacitracin were also potent bactericidal agents. Although Chloramphenicol inhibited growth of most strains effectively, it rarely exerted a killing effect; the majority of organisms tested were solidly resistant to tetracycline. Penicillin remains the most potent clinically useful bactericidal agent and was the antibiotic of choice against half of the strains in each group.

Organisms isolated from nasal carriers receiving antimicrobials were significantly more resistant than those from untreated carriers and the pattern of resistance bore no consistant relationship to the agent being administered. Furthermore, strains from infections were often more sensitive than those from nasal carriers. Sensitivity tests were of definite value to the clinician, and there was excellent correlation between recommended therapy and clinical response providing surgical drainage and other ancillary procedures were employed when indicated.

An Epidemic of Paracolon Providence in a Burn Unit

By *Count D. Gibson, Jr. and Boyd W. Haynes, Jr.* Richmond, Virginia.

An epidemic of *Paracolon providence* wound infections has developed in our burn unit in conjunction with the routine topical use of Bacitracin, Neomycin and Polymyxin, suspended in lactose powder. Between May, 1955 and August, 1956, 62 patients were thus treated. 568 burn wound cultures were obtained and the flora isolated in pure culture. The sensitivity of each organism to eight antibiotics in common use and to a mixture of Bacitracin, Neomycin and Polymyxin was determined semiquantitatively.

Observations were divided into five treatment periods, each of three months duration. During the first period, the usual burn wound flora was encountered. During the second period, an unusual coliform organism, identified as a paracolon of the *providence* group and sensitive only to Neomycin and the "banepo" mixture was isolated for the first time. During successive periods the incidence of the organism in burn wounds rose to a peak of 39% during the fifth period. Simultaneously, the frequency of *providence* strains resistant to Neomycin and "banepo" rose to 100%. At present, it is the only organism in the unit resistant to all antibiotics in common use. It has been detected on the fingers of the unit personnel and in air samplings from the dressing station and burn wards.

It is concluded that in a closed hospital unit, the large-scale use of antibiotics over body surfaces predisposes to the evolution of an unpredictably altered flora endowed with a high degree of antibiotic resistance.

Epidemiologic Studies of Pyoderma Neonatorum

By *Donald N. Wysham, Marie E. Mulhern, George C. Navarre, Gerald D. LaVeck, Alfred L. Kennan and W. R. Giedt.* Department of Medicine, Massachusetts General Hospital, Boston.

An epidemic of staphylococcal infections was studied in which 54 (46%) of the 117 infants observed developed clinical staphylococcal infections. To determine the source of the staphylococci causing this epidemic, daily cultures were taken from the infants in the nursery, from their mothers, and from fomites and the air in the nursery. Cultures were made repeatedly from the noses and hands of nursery personnel. The strains of staphylococci isolated were identified by both phage type and sensitivity to antibiotics. Over 5,000 cultures were made in the course of this study.

The majority of infections were caused by a single strain of staphylococcus, termed the epidemic strain. This strain was of phage type 52/42B/81, and resistant to penicillin, Streptomycin, and Tetracycline. The source of this strain was apparently within the obstetrical unit, for half of the infections occurred before discharge from the nursery, and 49 of the infants were colonized by this strain while in the nursery.

The mothers were not the source of the epidemic strain of staphylococci, for none of them were admitted to the hospital carrying this strain. Two nursery personnel were carriers of this strain. Both, however, had such limited contact with infants that they could not have maintained the epidemic.

Infants who were subclinically infected disseminated large numbers of staphylococci into the nursery environment, including the nursery air. Of 106 air samples taken in the nursery using a slit air sampler, 75% were positive for *Staph. aureus*.

It appeared that the epidemic strain of staphylococci was transmitted from infant to infant, probably through the air. The nursery personnel were of relatively little importance, either as the sources or as the transmitters of the strain of staphylococci causing this epidemic. Changes in nursery design or rooming-in appeared to be indicated to affect permanent control.

Hemorrhagic Brain Lesions in Chick Embryos Infected with Influenza Virus

By *Edward W. Hook and Robert R. Wagner*. Biological Division, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore.

Viral infections of the central nervous system produce both neuronal and vascular lesions. The coexistence of these lesions often makes it difficult to determine the primary pathogenetic effect of the virus. Infection of chick embryos with certain strains of influenza virus causes intense brain hemorrhages, apparently the result of direct viral action on vascular endothelium. The striking appearance and reproducibility of these lesions afford an ideal model for study of vascular reactions to viral infection. The present experiments were designed to study the evolution of the brain lesions, and the influence of age, route of inoculation and prior influenza virus infection.

The amount of virus in the brain increases rapidly after intravenous infection with neurotropic influenza virus (NWS). Small hemorrhages appear 30 hours after injection when the virus titer exceeds 1 million ID_{50}. Most embryos die in 36 to 48 hours with marked brain lesions. Microscopic examination reveals many dilated small blood vessels and multiple hemorrhages but no significant inflammatory reaction.

Extensive hemorrhages occur regularly in 14-day-old embryos; younger and older embryos are relatively resistant. Intravenous injection of small amounts of virus consistently produces marked brain lesions, but virus injected by other routes is far less effective. Prior allantoic infection with the same or another strain of influenza virus partially counteracts the lethal effect of subsequent intravenous challenge and almost completely prevents the development of cerebral hemorrhages. This protective effect differs from a classical interference phenomenon.

Epidemic Protracted Debility at Punta Gorda, Florida; An Illness Resembling Iceland Disease

By *David C. Poskanzer, Donald A. Henderson, E. Charles Kinkle and Seymour S. Kalter*. The United States Public Health Service and Department of Medicine, Duke University Medical School and Hospital, Durham, North Carolina.

Clinical and epidemiologic studies were undertaken of an obscure epidemic illness occurring in a small Florida community in the spring of 1956. The disease was characterized principally by fatigue, headache, nuchal pain, alteration in emotional status and mentation, nausea and vomiting, paresthesias and muscular pain. A prolonged and relapsing course was the most striking and distressing feature. The illness resembles in many respects a few other localized epidemics throughout the world, commonly termed "Iceland disease."

Twenty-one patients were selected for extensive clinical investigation; each received a physical, neurologic and mental status examination. In a house-to-house survey comprising 359 households to determine the actual local incidence of the illness, 62 cases were uncovered. It was estimated that among the 2,500 residents, at least 150, none younger than age 12, were afflicted.

Fatigue, headache and neck pain were reported by all patients in the selected study group. Tension, depression, and defects of memory with periods of mild confusion were found in most. The prolonged course of illness was apparent at a reexamination at five months. At that time only one of the patients was found to have become symptom-free. There were no fatalities.

The severity and number of symptoms contrasted with the paucity of physical findings. Ten patients presented evidence of difficulty in performance of simple calculation and retention tests. Isolated areas of mild to moderate impairment of sensation were found in 12. Spinal fluid examinations and electroencephalograms were normal. The disease appeared to be more severe in females and a disproportionate incidence of illness was experienced by medical and associated personnel. Epidemiologic evidence is consistent with a hypothesis of person-to-person transmission, but no agent was isolated from the throat swabs, stools, or blood or identified serologically by the usual laboratory procedures.

The study cannot define the symptom mechanisms of this illness. Disordered function of the nervous system was manifest, but the type and extent of such involvement remain unknown.

Changing Pattern of Staphylococcus Phage-Types Isolated from Drug-Induced Enteritis in Japan.

By *Saburo Nagaki, Makoto Saito, Keizo Ishii and Susumu Tomioka*. Ebara Hospital, Tokyo, Japan.

Staphylococcal enteritis was studied in bacillary dysentery patients treated with tetracycline-type antibiotics. Only 4 cases were observed in 1954

from which staphylococcus phage-type 42B was isolated. These patients were treated in the hospital. Three other phage-types appeared in ambulant patients. One ward outbreak with type 52/155/166 was observed in 1955, involving 15 persons in the hospital. During 1956, 12 cases were seen in the wards, all of them excreting staphylococcus phage-type 42B/29. This strain differed from type 52/155/166 by being susceptible to Chloromycetin. Both types were resistant to penicillin, Streptomycin and tetracycline. Staphylococcus enteritis after tetracycline treatment in Japan is accompanied by a scarlatiniform rash which is more evident in infections caused by type 42B/29. No staphylococcus types suspected of causing enteritis were isolated from 96 pus and 118 nose-throat cultures from ambulant patients. The carrier rate of the organisms found in enteritis was 15.2% among patients and 27.3% among staff members on the afflicted ward. Cross-infections could be traced. The microbes were excreted for 2 weeks and longer after the onset of the disease.

KIDNEY

The Renal Red Cell Shunting Mechanism and the Control of Renal Resistance

By *Lawrence S. Lilienfield and John C. Rose.* Cardiovascular Research Laboratory, Department of Medicine, Georgetown University Medical Center, Washington, D. C.

Recent studies suggest the existence of a renal red cell shunting mechanism. Pappenheimer and Kinter have proposed that this mechanism is a dynamic one, receiving its energy from the arterial pressure. These authors suggest that renal resistance is controlled by alterations in effective blood viscosity brought about by changes in the degree of red cell shunting.

Employing a single circulation indicator-dilution technic, simultaneous kidney transit times for red cells (Cr^{51}) and plasma albumin (I^{131}) were measured unilaterally in 22 sodium pentobarbital anesthetized dogs. Total renal vein blood flow was collected during the measurement period. In 12 of the dogs, studies were performed before and after induced alterations in arterial pressure. From the data, intrarenal red cell and plasma volumes, hematocrit, and resistance were calculated.

Despite two to tenfold increases in renal resistance, with marked prolongation of circulation times, no significant alteration in calculated intrarenal hematocrit was observed. The ratio of red cell to plasma mean transit times averaged .82 ± .06 (S.D.) at mean pressures averaging 118 mm. Hg, and .82 ± .08 at pressures averaging 57 mm. Hg. Intrarenal blood volume averaged 22 ± 4 ml./100 Gm. of kidney at control pressures and 16 ± 4 ml./100 Gm. at the lower pressures.

These studies indicate that the renal red cell shunting phenomenon is not pressure or velocity dependent. They suggest that shunting results from an anatomic rather than a dynamic mechanism. Under the conditions of this experiment, red cell shunting played no role in controlling renal resistance.

Impairment in Renal Concentrating Capacity with Heavy Exercise

By *Lawrence G. Raisz and Robert L. Scheer.* Medical and Radioisotope Services, Veterans Administration Hospital, and Department of Medicine, State University of New York, Upstate Medical Center, Syracuse.

The renal concentrating capacity during dehydration was studied in five healthy young men under conditions designed to produce acute changes in solute excretion within the "physiologic" range. Observations were begun in the morning after 18 hours of water deprivation and were continued for 6 hours. There was no prior restriction of dietary protein or salt. Urine was collected at 30 minute intervals. After three or four preliminary 30 minute periods, the effects of venesection (500–600 ml.), Mannitol infusion (0.3–0.6 mM./min.), 30 minutes of light exercise, and 30 minutes of heavy exercise were observed for four hours and compared with control observations. Total solutes in urine and plasma were determined by freezing point depression. Solute excretion was expressed as the osmolal clearance (C_{osm}), and the osmotic urine/plasma ratio was taken as the measure of concentrating capacity.

Heavy exercise produced a marked decrease in osmotic U/P ratio, C_{osm}, and creatinine clearance (C_{cr}). Before exercise the mean values were: osmotic U/P ratio 4.0, C_{osm} 1.9 ml./min. and C_{cr} 135 ml./min. After exercise osmotic U/P ratio fell to 2.8, C_{osm} to 0.5 ml./min., and C_{cr} to 92 ml./min. C_{osm} and C_{cr} returned to pre-exercise values in 2 hours or less. However, osmotic U/P ratio remained depressed and at 4 hours had returned to the pre-exercise value in only one subject.

The other procedures employed did not appear to alter the concentrating capacity other than by their effect on solute excretion. C_{osm} increased after Mannitol infusion, light exercise, and in some control experiments, and decreased or remained un-

changed after venesection. In these experiments there was a significant inverse correlation between C_{osm} and osmotic U/P ratio in each subject.

The acute impairment observed after heavy exercise indicates that the renal concentrating capacity is sensitive to transient metabolic or hemodynamic changes and can be used as a measure of intrinsic renal disease only under specially controlled conditions.

A Mechanism by Which Altered Distribution of Blood Volume Affects Renal Function

By *Herbert O. Sieker and Herschel V. Murdaugh, Jr.* Department of Medicine, Duke University School of Medicine and Veterans Administration Hospital, Durham, North Carolina.

Alteration of the normal distribution of blood volume has been demonstrated to vary renal excretion of water and electrolytes. This study was done to elucidate the mechanism by which changes in intrathoracic blood volume affect renal function. Continuous positive or negative pressure breathing for 30 minutes was used to decrease or increase intrathoracic blood volume. Twenty-two studies were done on 8 normal subjects. Urine volume, urine osmolarity, sodium and potassium excretion, and inulin and sodium para-aminohippurate clearance were determined in control and experimental situations. Positive pressure breathing (24–26 mm. Hg) was done during water, alcohol and osmotic diuresis. Negative pressure breathing (18–22 mm. Hg) was used with a constant fluid intake with and without the administration of Pitressin.

Positive pressure breathing during water diuresis decreased urine flow from 14.5 cc./min. (mean value) to 6.3 cc./min. (mean value) and with alcohol diuresis from 14.5 cc./min. (mean value) to 9.0 cc./min. (mean value). The antidiuretic effect lasted 43 minutes (mean value) during water diuresis and 22 minutes (mean value) during alcohol diuresis. No response was noted to positive pressure breathing during osmotic diuresis. Negative pressure breathing was associated with a three to fourfold increase in urine flow with the diuresis persisting 45 to 60 minutes (mean value). Pitressin completely blocked this effect. Glomerular filtration rate and effective renal plasma flow were decreased 10–25% at the time of maximum alteration in urine flow in both positive and negative pressure breathing. Measurement of solute and water clearance indicated that the oliguria or diuresis in these studies was due primarily to impaired or enhanced water clearance. Although sodium and potassium excretion remained unchanged, preliminary data indicate alteration of urea content of the urine secondary to changing urine flow.

The evidence suggests that pressure breathing and altered intrathoracic blood volume initiate alterations in renal function and urine flow through a hormonal mechanism, primarily an antidiuretic or antidiuretic-like hormone.

Active Renal Regulation of Urea Excretion in Man

By *Herschel V. Murdaugh, Jr. and Bodil Schmidt-Nielsen.* V. A. Hospital, Departments of Medicine and Zoology, Duke University, Durham, North Carolina.

Though urea is a major metabolic end product, it has long been considered to be handled passively by the kidney. Recent studies in camels, kangaroo rats, sheep and dogs have demonstrated an active renal regulation of urea excretion.

This study was performed to evaluate the mechanism of the renal excretion of urea in man. Hospitalized adult male subjects and active house staff volunteers were studied on a normal protein diet and a low protein diet (basic rice with added salt). Inulin, creatinine, urea and osmolar clearances were determined at high and low rates of urine flow.

The house staff volunteers were followed weekly with determinations of blood urea concentrations which decreased to approximately half the control value in one week, then remained relatively constant. The glomerular filtration rate was not significantly altered by the diet. After two weeks of low protein diet the urea excretion was less but the percentage of filtered urea excreted was not consistently changed. After five weeks, however, it was found that the percent of filtered urea excreted (urea clearance/inulin clearance) for any given rate of urine flow was one-fourth to one-half of that found during normal protein intake. This difference cannot be explained by changes in urine flow. The kidney appeared to conserve urea.

Urea was given orally to two subjects during low urea excretion. This caused the percentage of filtered urea excreted to increase as much as threefold within two hours. Again this change could not be accounted for by change in urine flow.

These studies show that urea excretion in man cannot be entirely passive as has been assumed hitherto. It appears that the regulation of urea excretion in man is similar to the mechanism found in other mammals.

Sodium Excretion in Renal Disease

By *Alvin E. Parrish and Mary F. Watt.* Mt. Alto Veterans Administration Hospital and George Washington University, Washington, D. C.

Sodium excretion by the normal kidney is only partially affected by glomerular filtration. Changes in the sodium load to the kidney result in increased sodium excretion (1) by decreased tubular reabsorption and (2) by increased sodium filtration due to

(a) a higher serum sodium or (b) an increased filtration rate, or both. Some investigators have felt that variations in filtration rate play a prominent role in maintenance of electrolyte balance under different salt loads in the normal kidney.

In the course of study of patients with renal disease confirmed by renal needle biopsy, 45 patients have been studied comparing the filtration rate (inulin clearance) with the sodium clearance. Fourteen patients without renal disease have also been studied.

In most of those with renal pathology there is a relation between inulin clearance and sodium clearance ($r = 0.725$, $p < 0.01$). This relation was not present in patients without renal disease; or in patients with extensive renal tubular pathology or a Tm PAH less than 10 mg./min, or acute glomerulonephritis.

Six patients with renal disease were given a sodium load intravenously (100 mEq. as 5% NaCl) and renal functions measured continuously for one hour using ten minute periods. The filtration rate changed little or none, serum sodium remained unchanged, filtered sodium remained unchanged or fell, excreted sodium and C_{Na} increased markedly within 10 minutes.

These findings suggest that under ordinary conditions in most patients with renal disease, sodium excretion may be more related to filtration than in normal individuals. When sodium loading occurs, the increased urinary sodium excretion results from a decreased tubular reabsorption alone without change in the filtration rate.

Osmotic Diuretic Treatment of Refractory Edema

By *Lionel M. Bernstein, Bernard Blumberg and Murray C. Arkin.* Department of Internal Medicine, Veterans Administration Hospital, Hines, Illinois.

Marked edema of cardiac failure, cirrhosis, and nephrosis occurs which becomes refractory to diuretics such as mercurials, ammonium chloride, carbonic anhydrase inhibitors, and probenecid. In general, these diuretics reduce sodium (and hence water) resorption by affecting the enzyme systems involved in the active tubular transport systems. In contrast with these, osmotic diuretics cause loss of sodium and water by a physical rather than by a metabolic effect on the kidney. The effect of osmotic diuretics is dependent upon filtration into the tubule of non-absorbable particles which cause retention of water in the isosmotic proximal tubules and presentation of amounts of water, sodium, and other electrolytes to the distal tubules in excess of their resorptive capacity. Thus, an osmotic diuretic effect should be present so long as significant glomerular filtration rates exist.

Mannitol, an inert, non-metabolizable, nontoxic hexose available for parenteral injection was used as a typical osmotic diuretic. Initial studies demonstrated that mannitol in doses of 50 to 100 Gm. caused a greater water loss, and as great a sodium loss per day as did 2 ml. of Thiomerin administered intravenously.

Mercurial diuretics exert their effects by interfering with active tubular transport mechanisms, the nonabsorbed sodium then behaving as osmotic diuretic particles. The combination of a physical osmotic diuretic (such as Mannitol) and a tubular transport blocking agent (mercurial diuretics as a prototype) would be expected to augment each other. In patients with refractory edema due to cardiac failure or nephrotic syndrome, Mannitol administered intravenously in doses up to 400 Gm. combined with mercurial diuretics effected urine volumes up to 5000 ml. greater, sodium losses up to 400 mEq. greater, and body weight losses up to 4 Kg. more per day than did the mercurial diuretics alone.

Dialysance and Pressure-Flow Relationships in the Disposable Twin Coil Artificial Kidney

By *Leonard B. Berman and George E. Schreiner.* Department of Medicine and the Renal Laboratory, Georgetown University Medical Center, Washington, D. C.

Unlike the rotating drum type of artificial kidney, the disposable coil is characterized by high resistance and the necessity for an arterial blood pump for adequate flow rates. At any pump speed, resistance of the coil determines flow and pressure and therefore is the fundamental determinant of filtration and dialysance. Early models of the disposable coil were found to have individually variable and excessive resistance sometimes requiring an inflow mean pressure in excess of 400 mm. Hg for flow rates over 200 ml./min.

Recent models have been tested in vitro and in vivo during ten clinical dialyses. Pressures at important sites were measured by a strain gauge transducer. Indicator-dilution curves were determined by serial sampling from the venous cannula after rapid injection of I^{131} labeled human serum albumin into the arterial cannula. Data thus obtained included pressures in multiple sites, blood flow rates under operating conditions, pressure-flow relationships, mean circulation times and coil blood volumes. Dynamic coil volumes which vary with pressure and flow have all been in excess of one liter.

Simultaneous arterial, venous and bath concentration of urea were used with flow rate measurements to determine dialysance. The dialysance fraction appears to be less than for the rotating Kolff-Merrill artificial kidney but is greater in proportion to the surface area of cellophane used. In clinical trials, the mean urea removal rate was 10 Gm./hr., and significant water removal was accomplished at pressures which produced no visible hemolysis.

Hemodynamic and Electrolyte Studies in Acute Glomerulonephritis

By *Yoshikazu Morita*. Wayne State University College of Medicine, Detroit.

Three patients with acute glomerulonephritis were studied at the height of, and during recovery from, edema. Each patient showed moderate generalized edema, mild dyspnea, cardiomegaly, increased venous pressure, and a normal arm-to-tongue circulation time.

Cardiac catheterization showed elevated pulmonary capillary and pulmonary artery pressures, and elevated pressures in the right heart chambers. Cardiac output, measured by the application of the Fick principle, using oxygen, was increased in all three, the cardiac index being 5.5, 6.6, and 4.4 L./min./M.^{2}, respectively, in patients A, B, and C. The arteriovenous oxygen differences were within the limits of normal in two, and low in patient B.

Patients B and C were studied by the balance technic for electrolyte shifts during spontaneous diuresis, which took 12 days in B and 4 days in C. Changes in extracellular fluid were calculated from chloride balance. There was a negative water balance of 6.3 L. in B, and 7.8 L. in C. Two-thirds of the total external loss of fluid came from the extracellular space. The nitrogen balance was −5.8 Gm. and −2.8 Gm., respectively, during the entire period. External sodium balance was negative, but the cells took up 107 and 121 mEq. of sodium, respectively. Potassium balance, corrected for N balance, was +53 and +31 mEq., respectively; magnesium balance, corrected for N balance, was +6.6 and +26.3 mEq., respectively.

It is concluded that hemodynamically, the congestive state of acute glomerulonephritis resembles the high output type of congestive heart failure. Electrolyte shifts during diuresis resemble qualitatively the changes which occur during diuresis in ordinary types of congestive heart failure.

Tubular Diluting Function in Nephrosis Before and After Prednisone Therapy

By *Mackenzie Walser and Jack Orloff*. The National Heart Institute, National Institutes of Health, Bethesda, Maryland.

Since the initial step in urinary dilution is presumably the reabsorption of sodium and anion in the distal tubule, patients with nephrosis and sodium retention might be expected to exhibit greater rates of solute-free water excretion, provided that adequate quantities of proximal fluid are delivered to the diluting site. If renal insufficiency is also present, the reduced number of functioning nephron units may result in a lower absolute rate of free water excretion. However, when computed as a fraction of the filtration rate, heightened free water excretion suggests enhanced diluting function per nephron unit and thus increased distal sodium reabsorption. Therefore, in order to compare tubular diluting function in nephrotics with that in normals, osmotic diuresis was superimposed upon sustained water diuresis and the urine flow was plotted as a function of the osmolar clearance, both calculated per 100 ml. GFR, determined simultaneously with inulin. Osmotic diuresis with Diamox tended to produce higher curves than with Mannitol in both groups, suggesting that the distribution of sodium reabsorption within the tubule differed. Compared to seven normal subjects, seven nephrotics with varying degrees of renal insufficiency (GFR 13 to 153 ml./min.) showed either normal (4) or enhanced (3) tubular diluting function. Higher minimal urinary osmolality in the patients with renal insufficiency was thus due to the obligatory excretion in them of a greater fraction of the filtered solute.

Prednisone therapy raised filtration rate in six patients, and was associated with remissions in two patients. In one of these, filtration rate rose 250%; in both, tubular diluting function was increased. In the patients who did not respond, diluting function was not affected by Prednisone, nor by prolonged administration of albumin.

Metabolic Studies in the Nephrotic Syndrome

By *John D. Blainey*. Department of Medicine, University of Illinois, Chicago, and Department of Experimental Pathology, University of Birmingham, England.

Strongly positive nitrogen balance has been observed in patients with the nephrotic syndrome for prolonged periods while on treatment with high protein diets.

In an attempt to correlate these observed nitrogen gains with other metabolic data and with changes in body weight, metabolic balances of nitrogen, potassium, sodium, chloride and water have been measured over periods of several months in 5 adult patients with the nephrotic syndrome.

Nitrogen and potassium balances were strongly positive throughout. Early in treatment potassium was retained in considerable excess of nitrogen in some patients; after prolonged high protein feeding the potassium nitrogen ratio approached the normal value of 3 to 1.

During periods of edema formation or diuresis, changes in body weight were largely accounted for by gains or losses of fluid. A close relationship was observed between water balance and sodium or chloride balance which indicated that this fluid had the composition of extracellular fluid with respect to these ions, except for a short time at the beginning of diuresis when water was lost in excess of electrolytes.

Correction of the observed changes in body weight for gains or losses of edema fluid indicated that early in treatment nitrogen was retained con-

siderably in excess of that expected from the normal ratio of nitrogen to protoplasm weight. As repletion of the body protein stores proceeded, the positive nitrogen balance was accompanied by the expected gain in body weight.

The relationships described were similar when diuresis occurred spontaneously or when it was induced by cortisone therapy.

Pathophysiologic Changes in the Adult Fanconi Syndrome

By *Robert E. Dedmon, J. Herbert West and Theodore B. Schwartz.* Department of Medicine, Presbyterian-St. Luke's Hospital and University of Illinois, Chicago.

Studies were designed to elucidate more clearly the abnormal tubular transport mechanisms associated with the adult Fanconi syndrome. Detailed observations have been made on two adults who satisfy the proposed criteria for this disorder, i.e., glycosuria, phosphaturia, and amino-aciduria with normal blood levels of these metabolites, plus osteomalacia with fractures and pseudo-fractures.

The first patient, a 51-year-old male, has, in addition, severe parenchymatous liver disease and a bleeding tendency resulting from thrombocytopenia and deficiencies in prothrombin and Factor V. Electrophoretic analysis revealed high gamma peaks in both serum and urine. Rectangular crystals have been demonstrated repeatedly in aspirated bone marrow. The diagnosis of multiple myeloma was suggested, but could not be substantiated. The second patient is a 53-year-old female who has mild azotemia. She has noted a reduction in stature, and skeletal roentgenograms have revealed the presence of more than 50 pseudo-fractures.

In both patients phenosulphonphthalein excretion was markedly impaired and persistent mild metabolic acidosis was present. There were impressive increases in urinary excretion of glucose, phosphate, uric acid, creatine, alpha-amino nitrogen, free glycine, and chromatographically partitioned free amino acids, while plasma concentrations of these metabolites were either normal or decreased. These excessive urinary losses reflect a striking tubular reabsorptive defect since there was a considerable reduction in glomerular filtration rate.

Renal tubular reabsorption of phosphate (TRP) was greatly depressed and could not be enhanced by the intravenous infusion of calcium. However, an even greater reduction in TRP could be provoked following parathyroid stimulation resulting from a Na-EDTA-induced hypocalcemia. Intracellular levels of free glycine and alpha-amino nitrogen of biopsied muscle were appreciably higher in the female patient than in a normal subject.

It is postulated that the pathophysiologic changes resulting from chronic renal disease suffice to account for the clinical manifestations and that variants of this syndrome may evolve in any patient in whom renal tubular impairment exceeds any concurrent glomerular insufficiency.

Relationship Between Pyelonephritis and Bacterial Counts in Urine: Autopsy Study

By *Richard A. MacDonald, Howard Levitin, G. Kenneth Mallory and Edward H. Kass.* Mallory Institute of Pathology, the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital; the Department of Pathology, Boston University School of Medicine and the Department of Medicine, Harvard Medical School, Boston. (Aided by a grant from the National Institutes of Health.)

Difficulties in the diagnosis of pyelonephritis have led to the use of bacterial counts of urine as a means for distinguishing true bacteriuria from contamination. Bacterial counts greater than 10^5/ml. of urine generally indicate multiplication of the organisms within the urinary tract and substantial numbers of asymptomatic patients with such true bacteriuria can be found. The relationship of bacteriuria to pathologic lesions of the urinary tract was explored in the present study.

Bacterial counts of bladder urine were performed in 100 unselected adult autopsies and correlated with pathologic findings. Forty patients had $>10^5$ bacteria/ml. of urine, 7 had 10^4–10^5/ml. and 53 had none. Active pyelonephritis was found in 14 of the 40 with $>10^5$ bacteria but only in 3 of the remaining 60; each of these 3 had received intensive antibacterial therapy before death. Three instances of acute cystitis without renal involvement were found among those with 10^5 bacteria/ml.

Healed pyelonephritis occurred in 18 patients and bore no relationship to bacterial counts in the urine. A correlation was found between catheterization and bacteriuria at autopsy, as well as with pyelonephritis. Pyuria, azotemia, granular casts, albuminuria, etc. were not reliable indices of the presence or absence of bacteriuria or pyelonephritis. No relationship to hypertension was demonstrable.

The finding of true bacteriuria at autopsy correlates well with the presence of pyelonephritis and suggests that such a relationship may also obtain during life.

Five-Year Experience with Renal Biopsy

By *Alvin E. Parrish, Mary F. Watt and John S. Howe.* Mt. Alto Veterans Administration Hospital and George Washington University, Washington, D. C.

Since the initial report of Iversen and Brun, renal needle biopsy has become increasingly popular, using a number of technics and instruments. A review of those patients biopsied with a Turkel needle using the technic previously described shows that

renal tissue was obtained in 60% of instances in the first 50, in 87% in the next 100, and 94% in the last 100. Unsatisfactory biopsies (inadequate or ruined in preparation) amounted to 6% of the first 50; 9% of the next 100, and 11% of the last 100. Significant complications have consisted of bleeding, either retroperitoneally (4) or into urine (1), a total of 1.7% of 285 biopsies. Bleeding has required surgical intervention once. There have been no fatalities.

All types of renal disease have been biopsied and the complications have occurred only in hypertensive patients. Two of the 4 patients bleeding retroperitoneally had malignant nephrosclerosis. A tissue diagnosis may be made in 90% or better of satisfactory (10 or more glomeruli) biopsies. Contraindications are considered to be (1) bleeding diathesis and (2) unilateral kidney.

Renal Cortical Necrosis

By *Jacques D. Wells, E. Gordon Margolin and Donald I. Radin.* University of Cincinnati College of Medicine, Cincinnati.

Twenty-one necropsied cases of renal cortical necrosis occurring since 1953 have been reviewed at the Cincinnati General Hospital. In our recent experience this disease represents 20% of all cases of acute renal failure.

Only 3 of the 21 cases were associated with abruptio placentae. There were 3 cases with extensive body burns, 3 with gastrointestinal hemorrhage, and 3 with peritonitis. Eight of the remaining 9 cases were associated with a variety of illnesses. One patient had no known pre-existing disease.

Those who lived for a sufficient period followed a course difficult to differentiate from that of the oliguric phase of other types of acute renal failure. Of the 15 patients in whom accurate urinary outputs were determined, 12 had total anuria for 1 or more days. The 3 patients without total anuria at any time had the least amount of renal cortical necrosis at necropsy. Fourteen of these 15 cases had less than 50 cc. of urine daily for at least half of their course of renal failure. Hematuria occurred in 14 cases in whom urine was examined. Hypotension preceding recognition of renal failure occurred in 15 of 18 cases. Hypertension during renal failure occurred in 7 of 21 patients. Twelve patients in whom the blood pressure was measured the last few hours of life had shock terminally. At least 12 patients had abdominal or flank pain.

Seventeen patients received whole blood and 3 received plasma during observation. In no instance was a transfusion reaction noted. All patients were treated with antibiotics, 5 with steroids, 14 with pressor drugs and 8 with hemodialysis.

Renal cortical necrosis in our experience has become a relatively frequent cause of acute renal failure and is strongly suspected when total anuria and prolonged severe oliguria are observed.

The Determination of Clinically Significant Urinary Cultures By Molecular Membrane Filtration

By *R. E. Ritts, Jr., Frances H. Mao and C. B. Favour.* The Departments of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston. (Aided by a grant from the Upjohn Co.)

A study has been made to correlate clinical urinary tract disease with urinary bacteriology by a new technic, molecular membrane filtration (MF), and by pour plates (PP) in order to find what number of colonies per volume of urine constitutes a significant urinary tract infection. Spun sediments of catheterized urine samples from 66 patients were examined microscopically and the bacilluria estimated on a scale of 5+. Measured quantities of urine from 0.0001 to 1.0 cc., inversely proportional to the gross estimation, were incorporated in 15 cc. of tryptic digest broth for urine pour plates. Similarly, duplicate urine samples of 10^{-3} to 10 cc. were made to 20 cc. in doubly distilled water and passed through MFs which were then sterilly transferred to nutrient pads for incubation.

When there was no growth on the PP there was no growth on the MF. Patients submitting these samples were without urinary infection. Conversely, when the PP were "loaded" (>100,000 colonies per cc. of urine) the MF were similarly overgrown, even when as little as 0.00001 cc. of urine was passed through the MF. These specimens were from patients having clinically obvious urinary tract infections. When bacilluria was between these extremes, the MF showed significant growth at 1–5 dilutions higher than the PP, and was ready for examination and identification in as little as six hours after inoculation. Further, when the PP indicated 5–1,000 colonies per cc. of urine, suggesting contamination, there was poor correlation with clinical findings. In this range the MF often showed significant bacilluria (>1,000 colonies per cc. of urine) and gave good clinical correlation. Parallel in vitro sensitivity studies on the organism isolated by the two methods showed no significant difference.

The rapid growth of organisms in minute quantities of urine on molecular membrane filters appears to indicate the usefulness of this technic in questionable urinary tract infections.

Laboratory and Clinical Evaluation of the Coil Kidney

By *Robert Meyer, Ralph Straffon, Searle B. Rees, Warren R. Guild and John P. Merrill.* Boston.

The several types of artificial kidney clinically available for extracorporeal dialysis differ principally in the kinetics of contact between the patients' blood and the dialyzing fluid. The Kolff Disposable or Coil Kidney requires both blood and dialyzing fluid to be in motion by means of two pumps and a

unit of twin cellophane envelopes separated by fiber glass screen and coiled around an 11 cm. can. This report contrasts the Kolff Coil Kidney with the Kolff-Brigham Rotating Kidney.

Results indicate that clearances of measurable diffusible substances are comparable in both units. The increased hydrostatic pressure of the blood within the coil essential for the operation of this kidney results in the ultrafiltration of 250–500 cc. of water per hour of dialysis. This was determined by weight loss of the patients and by I^{131} albumin concentrations.

The Kolff Coil Kidney has the same major disadvantages as the rotating model; i.e., it is not portable and requires a staff of highly skilled professional personnel.

We conclude that the Kolff Coil Kidney is for clinical purposes as efficient a dialyzing unit as the Kolff-Brigham Rotating Kidney. The Kolff Coil Kidney has the additional advantages of ultrafiltration, disposability of parts requiring sterilization, and simplicity of assemblage. The entire unit with two pumps and a 100 to 120 liter tank to contain the dialyzing fluid is comparatively inexpensive and requires minimal maintenance.

A Six-Year Follow-up Study of Hereditary Interstitial Pyelonephritis

By *G. T. Perkoff, C. A. Nugent, F. E. Stephens and F. H. Tyler.* Salt Lake City, Utah.

In 1951 we reported a large family in which pyelonephritis was inherited as an incompletely sex-linked dominant. Severely involved males had associated nerve deafness. Since 1951, other investigators have reported a different family with hereditary pyelonephritis. Another group has described a syndrome of hereditary hematuria, renal functional impairment and nerve deafness. Because of the similarities in these reports and better methods now available to us, the original family has been restudied.

Physical examination and urinalysis were performed on 161 patients, 71 of whom had not been seen earlier. Ninety-three audiograms were done. Five percutaneous renal biopsies and an additional autopsy (three in all) have been performed.

Most of the subjects were asymptomatic. Abnormalities of the urine sediment were present in 45. Pyuria, hematuria and proteinuria were observed in that order of frequency. The additional autopsy showed severe interstitial pyelonephritis, as had the previous studies. Two renal biopsies in affected young girls revealed a few hyalinized glomeruli, minimal interstitial infiltration and red cells in the tubules. Two biopsies in adult women showed small areas of interstitial scarring, tubular atrophy, mild inflammatory infiltration and a few hyaline glomeruli. Biopsy done in a woman who later died in uremia showed advanced changes compatible with pyelonephritis. The pattern of inheritance was consistent with that of an incompletely sex-linked dominant.

The presence of hematuria, particularly in young patients, the progressive pathologic changes with age, plus the nerve deafness, make it probable that this syndrome is closely related to "hereditary hematuria." It would appear that all of these patients inherit an as yet unidentified renal abnormality which, in our cases, leads to pyelonephritis. These studies may have important implications in the pathogenesis of apparently sporadic pyelonephritis.

LIVER

Quantitative Evaluation of Hepatic Uptake and Release of Radioactive Rose Bengal

By *Edwin Englert, Jr., Belton A. Burrows and Franz J. Ingelfinger.* Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals and the Boston University School of Medicine, Boston.

Following injection of rose bengal labeled with I^{131} in normal subjects, radioactivity in the liver increases rapidly and then diminishes slowly. The rise has been ascribed to hepatic "uptake" and the fall to hepatic "excretion," but dye in blood, gallbladder, biliary tree and duodenum and backscatter from these sources could modify these interpretations.

The validity of radioactive rose bengal "uptake-excretion" curves obtained by external counting was studied in 14 cholecystectomized patients. Bile was aspirated constantly from common duct or duodenum. "Uptake" and "excretion" were exponential functions whose rates were derived by graphic analysis as halftime of "uptake" and halftime of "excretion" and were compared to the rate of removal of dye from the plasma and to the rate of accumulation of dye in the bile.

Dye in the hepatic blood volume may be estimated from the dose-response relationship of I^{131}-albumin to hepatic counting and the plasma removal plus hepatic "uptake" rates of rose bengal. After 10 minutes, this source did not interfere with interpretation of the hepatic "uptake" curve.

Hepatic "uptake" rate was closely correlated with plasma removal rate ($r = +0.9$) and the slope of the regression approximated a 1:1 relationship. Presumably because of incomplete bile collections, hepatic "excretion" rate did not relate exactly to

the rate of dye accumulation in the bile. In subsequent tests, sulfobromophthalein, a relatively nonabsorbable substance was used to control the completeness of bile collection and to provide a correction factor. In such corrected bile collections the rate of accumulation of radioactivity correlated with the hepatic "excretion" rate obtained by external counting ($r = +0.9$). This regression slope approximated 1.0.

Correlation does not prove causation but the data suggest an intimate 1:1 relationship between rates of hepatic "uptake" and "excretion" by external counting and rates of plasma removal and bile accumulation, respectively, of administered rose bengal.

Clinical Studies With Radioiodine Rose Bengal Dye

By *James A. Wood and Donald R. Korst.* Radioisotope Service VA Hospital and Department of Medicine, University of Michigan, Ann Arbor.

Radioiodine-labeled rose bengal dye has been used in a group of normal subjects and in a group with various types of liver disease to establish patterns of hepatic concentration and bile excretion. External scintillation counting is used to follow liver concentration (posterior liver position), plasma clearance (precordium), gall bladder concentration (anterior liver), and excretion into the bowel (lower abdomen). Counting over the posterior liver after placing 20 μc. of I^{131} in the gall bladder of a cadaver resulted in less than 5% of the counts obtained over the anterior liver position.

An estimated 10–18% of the administered dose is absorbed after oral injection and about 15–28% is reabsorbed after T-tube administration, indicating some entero-hepatic circulation. A hepatic uptake curve is obtained during a 2 hour period and followed by a 24-hour count. Gall bladder concentration is indicated by the difference of anterior and posterior liver counting. A ratio of posterior liver to lower abdomen counting indicates adequacy of bile excretion into the intestine. Repeated liver concentration curves in a dog before and after common bile duct ligation failed to show any change in hepatic concentration of the dye, and there was more gall bladder concentration of radioactivity after ligation.

Six patients with Laennec's cirrhosis or postnecrotic cirrhosis showed a poor initial hepatic concentration and a gradual uptake over a 24-hour period. A patient with chlorpromazine jaundice showed a normal uptake and slightly retarded excretion pattern. Two patients with extra hepatic obstruction of a month or more duration demonstrated almost normal hepatic concentration and very slow excretion during the 24-hour period. It would appear from these studies that it may be possible to differentiate between intra- and extrahepatic obstruction using the isotope-labeled dye.

Radiothyroxine Turnover Studies in Liver and Biliary Tract Disease

By *Kenneth Sterling and Robert B. Chodos.* Department of Medicine, State University of New York, Upstate Medical Center, and Radioisotope Service, Veterans Administration Hospital, Syracuse.

The rate of removal of I^{131}-labeled thyroxine from the circulation was studied in 11 cases of Laennec's cirrhosis and 7 cases of other diseases of the liver and biliary tract. Deviations from the normal turnover of thyroid hormone were anticipated in view of the role of the liver in the metabolism of various hormones including thyroxine.

The pool of extrathyroidal organic iodine showed small but significant enlargement in the abnormal group (mean 735 μg. I) as compared with the normal controls (mean 488 μg. I). In three patients with cirrhosis the turnover rate was slower, and in four it was faster than the normal mean. The mean of the degradation rates (representing the products of the pools and turnover rates) was slightly but significantly higher (76 μg. I per day) than the normal control mean (51 μg. I per day). The differences observed were not clearly related to the presence of jaundice, ascites, or to any specific disease or functional disorder of the hepatobiliary system.

The magnitude of the elevations of pool size and degradation rate were not considered likely to have clinical significance.

Erythrocyte Dynamics in Liver Disease

By *Charles A. Hall.* Department of Medicine and Medical Research Laboratory, V.A. Hospital, Albany, New York.

While it has been clearly demonstrated that some patients with liver disease show a shortened red cell life span, it appears that other erythrocyte abnormalities must be present, for the patients reported in the literature did not compensate for the short life span as do certain patients of other types with more severe hemolytic processes. The present study was undertaken to measure cell life span, red cell mass, and the effective rate of erythropoiesis in patients who were relatively stable as regards their chronic liver disease.

Erythrocyte life span and red cell volume were determined in 14 patients by Cr^{51} methods. Daily erythrocyte production was calculated from the data. It was found that (1) the red cell volumes ranged from subnormal to above normal, and did not correlate well with the hematocrit; (2) half of the patients had shortened cell life spans, the shortest being 63 days; (3) six compensated for a shortened cell life by increasing erythrocyte production; (4) three had subnormal rates of erythropoiesis and did not maintain a normal red cell mass; (5) a comparison of mean erythrocyte diameter and rate of ery-

thropoiesis failed to demonstrate any correlation. All patients had macrocytosis.

The data suggest that in chronic liver disease there is an abnormality of erythropoiesis. In some instances this is of sufficient magnitude to prohibit maintenance of a normal red cell mass or compensation for a decreased cell life span, but in other instances it is not. It was demonstrated that the macrocytosis of liver disease is not due simply to increased rate of erythropoiesis, but probably reflects a more fundamental disturbance.

Diuretic Response to Water Administration in Patients with Infectious Hepatitis

By *Solomon Papper, Harold W. Seifer and Lawrence Saxon.* Medical Service, Boston V. A. Hospital, and the Departments of Medicine, Boston University School of Medicine and Tufts University School of Medicine, Boston.

Although reports in the literature describe impairment of water excretion in patients with infectious hepatitis, the nature of the abnormality has not been delineated. Accordingly, the response to water loading has been investigated in 9 patients with acute infectious hepatitis. Total solute, sodium, potassium and chloride excretion, and endogenous creatinine clearances have been determined. A water load of 20 ml./Kg. body weight was administered and sustained, by the oral route in one test and by the intravenous route (4% invert sugar) on another occasion in 6 patients, by the intravenous route alone in two and orally alone in one. Attainment of "maximal" urine flow which varied from 9.4 to 28.0 ml./min. was not delayed. In three instances the diuretic response to intravenous loading was slightly greater than when the water was taken orally. Urine osmolality at the height of diuresis was low, ranging from 38–77 mOsm./l. Total solute excretion varied between 460 and 1430 micro-Osm./min. The rate of sodium excretion during "maximal" diuresis varied from 13 to 290 micro-Equiv./min. Endogenous creatinine clearances were within normal limits.

One patient whose initial diuretic response was 12.7 ml./min. with an osmolality of 36 mOsm./l. had an unfavorable course and died of acute hepatic necrosis. Two days before death the maximum flow achieved was 7.5 ml./min. and the lowest concentration was 101 mOsm./l. Creatinine clearance was normal during both tests.

Thus, in all 9 patients studied during the acute phase of infectious hepatitis, a normal diuretic response to water administration was demonstrated. Minimal impairment was observed in one gravely ill patient restudied just prior to death.

Determinations of Iron, Mucoprotein, Transaminase and Cholinesterase in the Serum in the Differentiation of Primary Biliary Cirrhosis, Secondary Biliary Cirrhosis and Cholestatic Hepatic Disease

By *Martin S. Kleckner, Jr.* Department of Internal Medicine, Yale University School of Medicine, and Hartford Hospital, Hartfold, Connecticut.

Previous investigations have disclosed that the conventional hepatic function tests are unreliable in differentiating conclusively primary biliary (cholangiolitic) cirrhosis from secondary biliary cirrhosis or cholestatic hepatic disease (extrahepatic biliary obstruction). On the other hand, needle biopsy of the liver discloses that the hepatic lesions of primary biliary cirrhosis are not dissimilar to those of secondary biliary cirrhosis, and that this technic is hazardous in patients with cholestatic hepatic disease. Consequently, surgical biliary exploration and operative cholangiography are necessary to distinguish these conditions. This study included three cases of primary biliary cirrhosis, two cases of secondary biliary cirrhosis, and three cases of cholestatic hepatic disease. Marked hepatic insufficiency was not demonstrated in any case. One patient with primary biliary cirrhosis disclosed cutaneous xanthomatosis and had elevations of the blood cholesterol and phospholipids. These patients were studied by serial determinations of iron, mucoprotein, transaminase (glutamic-oxaloacetic), and cholinesterase of the serum.

No abnormalities in the serum iron were noted in the three conditions. The serum mucoprotein was significantly elevated in all cases of cholestatic hepatic disease and secondary biliary cirrhosis, until biliary obstruction was relieved, and in two cases of primary biliary cirrhosis. The serum transaminase was normal in all of the cases excepting one case each of primary and secondary biliary cirrhosis. The serum cholinesterase was normal in all of the cases excepting one patient each with primary and secondary biliary cirrhosis. These newer hepatic function tests do not differentiate primary from secondary biliary cirrhosis or from cholestatic hepatic disease. Serial determinations may be of value in assessing biochemically the progression of these types of hepatic disease to hepatic insufficiency.

Significance of Serum Transaminase Activity in the Differential Diagnosis of Jaundice in the Newborn Infant

By *Simon Kove, Stanley Goldstein and Felix Wróblewski.* Department of Pediatrics, New York University College of Medicine, Children's Medical Service, Bellevue Hospital, and Sloan-Kettering Institute and Department of Medicine, Memorial Center for Cancer and Allied Diseases, New York.

Jaundice in the newborn infant of undetermined origin is a problem which is not uncommonly encountered nor easily resolved. Early diagnosis is

important in order to minimize morbidity and prevent possible mortality. In newborn infants, in contrast to adults, the history, physical examination and available laboratory technics often fail to supply adequate information from which an etiologic diagnosis may be made.

The simultaneous determination of serum glutamic oxaloacetic transaminase (SGO-T) activity and serum glutamic pyruvic transaminase (SGP-T) activity offers a simple laboratory procedure which may be of significant diagnostic assistance in cases of jaundice of the newborn.

Previous investigations by the authors have demonstrated that in newborn infants the normal physiologic range of enzyme activity are levels up to 160 units for SGO-T and 80 units for SGP-T. This is in contrast to a range of activity up to 40 units for both serum enzymes in normal adults. The levels of activity in newborn infants with physiologic jaundice, regardless of the severity of the hyperbilirubinemia, remained within the physiologic neonatal range. Likewise, in infants with hemolytic disease of the newborn, the serum enzyme activity remained within the range obtained for normal newborn infants.

Preliminary observations indicate that elevation of enzyme activity occurs in extra-hepatic biliary obstruction, but the level remains below approximately 400 units. In acute hepatitis the enzyme activity usually rises above 400 units.

Thus, in the differential diagnosis of neonatal jaundice of unknown origin, an elevated level of serum enzyme activity would exclude physiologic jaundice, hemolytic diseases, and generalized infections causing hemolysis as possible etiologic factors. Elevated levels of activity of 160 to about 400 units would apparently favor the diagnosis of extrahepatic biliary obstruction. Enzyme activity above 400 units, with SGP-T activity usually reaching a higher level than that of SGO-T, would appear to indicate the presence of acute hepatitis.

The Significance of Alterations in Serum Enzymes in the Differential Diagnosis of Jaundice

By *Felix Wróblewski*. Sloan-Kettering Institute and Department of Medicine, Memorial Center for Cancer and Allied Diseases, New York.

Although a battery of liver function blood tests may at times be necessary to help define the etiologic explanation of hyperbilirubinemia in the jaundiced patient, in most instances the laboratory information will suffice if limited to the determination of the serum total bilirubin, serum alkaline phosphatase and serum transaminase.

Extrahepatic biliary obstructive jaundice is readily differentiated from that due to acute homologous serum and infectious hepatitis by the fact that the serum alkaline phosphatase is usually higher in the former than the latter. As a rule, in the initial or increasing icteric phase of acute hepatitis both serum transaminases are well over 400 units, while in obstructive jaundice the serum transaminases are usually below 400. In both instances, SGP-transaminase is greater than the simultaneously measured SGO-transaminase activity. In addition, the serial alterations in the serum enzymes in obstructive jaundice and jaundice associated with acute hepatitis are readily distinguishable. The alterations in serum total bilirubin and serum enzymes in a patient with acute extrahepatic obstructive jaundice and in a patient with acute hepatitis are quantitatively and serial distinct in the two types of jaundice. Although toxic hepatitis due to drugs may mimic the serum enzyme alterations seen in obstructive jaundice especially as in the case of chlorpromazine, when the alkaline phosphatase may become appreciably elevated, hepatitis due to hepatoxic agents may be readily distinguished from obstructive jaundice and acute hepatitis. When the toxic insult to the liver is stopped by the discontinuance of the administration of hepatoxic agent, the serum transaminases begin to fall at once toward normal, even though the serum bilirubin and/or serum alkaline phosphatase may transiently remain unchanged or even increase.

Intrahepatic carcinoma and lymphoma associated with jaundice present serum enzyme changes similar to those observed in cases of cirrhosis. However, in most instances of active Laennec's cirrhosis with hyperbilirubinemia the serum alkaline phosphatase is normal or only slightly elevated, while in most cases of intrahepatic malignant neoplasm with jaundice, the alkaline phosphatase is appreciably elevated above normal. Postnecrotic cirrhosis, unlike Laennec's, may be associated with an elevated serum alkaline phosphatase activity and, consequently, may present serum enzyme alterations which mimic those observed in intrahepatic neoplasia with jaundice. Hemolytic jaundice in the adult is usually readily distinguishable from other causes of jaundice; in most instances the serum enzymes remain normal except for transient and slight elevations in SGO-transaminase with little or no alteration of SGP-transaminase above the normal range.

Most types of surgically amendable jaundice, extrahepatic biliary tract obstructive jaundice, can be distinguished laboratory-wise from medical types of jaundice by the characteristic alterations in serum enzymes. In obstructive jaundice, serum alkaline phosphatase is elevated usually to a level greater than 10 units. SGP-transaminase is increased to a greater extent than the simultaneously measured SGO-transaminase, the former usually to levels less than 400 and the latter less than 300 units. In all the types of medical jaundice other than acute hepatitis, SGO-transaminase values are greater than simultaneously determined SGP-transaminase activity. In the case of acute hepatitis to the increasing icteric phase when SGP-transaminase is greater than SGO-

transaminase, the values of the serum transaminases are greater than 600 and 500 units, respectively.

Comparative Value of Serum 5-Nucleotidase and Alkaline Phosphatase in the Differential Diagnosis of Jaundice

By *Irving I. Young.* Department of Medicine, Wayne State University College of Medicine, and City of Detroit Receiving Hospital, Detroit.

Elevated serum 5-nucleotidase values, above 3.2 units/100 ml., have so far been observed only in subjects with hepatobiliary diseases. 89.8% of 88 subjects with acute hepatitis and portal cirrhosis had initial values below 10 units. 10.2% had levels between 10 to 18 units and no initial value was above 18 units. In occasional individuals with hepatitis, 5-nucleotidase levels rose to 18 to 24 units in the second week of illness. Simultaneously determined alkaline phosphatase values were greater than 15 Bodansky units in one case and between 9 and 15 BU in 26.2%.

Initial 5-nucleotidase levels in 39 subjects with extrahepatic biliary tract obstruction were above 18 units in 66.6%, and above 15 BU of alkaline phosphatase activity in 61.5%. 28.2% had 5-nucleotidase levels between 10 and 18 units, whereas 30.7% had alkaline phosphatase values between 9 and 15 BU.

Advanced hepatocellular damage with impending or frank coma was consistently associated with normal 5-nucleotidase values in 13 cases, but normal alkaline phosphatase levels in only 4. Primary or metastatic carcinoma of the liver was associated with elevated 5-nucleotidase and alkaline phosphatase levels in 13 of 14 cases, but in 9 individuals with isolated neoplastic involvement of bone and high alkaline phosphatase levels, serum 5-nucleotidase was normal.

The serum 5-nucleotidase determination is therefore at least as sensitive as the alkaline phosphatase in detecting the presence of biliary tract obstruction, and is more selective because values are not increased in diseases of bone associated with increased osteoblastic activity.

A New Method For Rapid Measurement of Hepatic Blood Flow and Portal Circulation Times Employing Radioactive Indicator Dilution Technics

By *Stanley Reichman, Richard Gorlin, John P. Storaasli and William D. Davis, Jr.* U.S. Naval Hospital, Portsmouth, Virginia.

A new method for measurement of hepatic blood flow using indicator dilution technics is based upon rapid intrasplenic injection of 1 cc. of radioactive iodinated serum albumin (30–50 μc). The curve of radioactivity, recorded in 34 patients, either by continuous recording equipment attached to a scintillation counter placed externally over the liver or from multiple hepatic vein blood samples drawn through a previously inserted catheter (15 patients), reflects the manner in which tracer substance is diluted in the hepatic vascular bed after it leaves the spleen, and can be used to calculate hepatic blood flow. The flow values obtained from suprahepatic curves and from hepatic vein sampling method correlated well, ranging from 0.5–1.1 L./min./M.^{2}, and were comparable with 5 flows estimated by BSP technic. No complications from splenic puncture were encountered nor was intrasplenic residuum of tracer substance significant.

This technic also permitted calculation of circulation times from spleen to liver, through liver, and from spleen to periphery. In the latter case a second scintillation counter was placed over the brachial artery. No significant differences in circulation times from spleen to liver and through liver were noted in normals as compared to cirrhosis. Premature peripheral arrival time was noted in two of three cirrhotics with proved varices. Particular applicability was found in studies showing rapid changes, i.e., decrease as seen with exercise (1 patient) and following pituitrin administration (2 patients), increase following food ingestion (1 patient), and following surgery in a patient with constrictive pericarditis when cardiac output increased. Five patients having advanced cirrhosis with and without varices exhibited lower flows (av. 0.63/L./min./M.^{2}) than did 16 normals (av. 0.81/L./min./M.^{2}), though statistical evaluation was not considered accurate because of small numbers in this study. Eight patients with active hepatitis (av. 0.89/L./min./M.^{2}) showed no significant difference in flows after full convalescence (av. 0.80/L./min./M^2.).

Conversion of "Indirect" to "Direct" Reacting Bilirubin by Human and Rat Liver in vitro and Studies of a Defect in Bilirubin Conjugation in Constitutional Hepatic Dysfunction (Gilbert's Disease)

By *Irwin M. Arias and Irving M. London.* Department of Medicine, Albert Einstein College of Medicine, New York.

Billing and Lathe, and Schmid have demonstrated that "direct reacting" bilirubin (DRB) is the glucuronide of bilirubin which, unconjugated, is "indirect reacting" (IRB). Dutton and Storey had previously described a rat liver homogenate capable of conjugating glucuronic acid with various acceptor compounds; such conjugation was increased by the addition of a factor in a boiled liver extract which was later identified as uridine diphosphate glucuronic acid (UDPGA). Strominger et al. have found an enzyme in liver microsomes which transfers glucuronic acid from UDPGA to morphine and orthoaminophenol.

In the studies reported here, the conversion of

IRB to DRB is shown to occur in vitro in a system comprised of (1) IRB, (2) a homogenate of freshly obtained rat or human liver, and (3) an extract prepared from rat or human liver. Incubation of IRB with the liver homogenate alone resulted in a slight formation of DRB. On addition of the human or rat liver extract, the formation of DRB was increased many fold. After fractionation of the homogenate, the activity was found primarily in the microsomal fraction.

Chromatography of the two resulting azopigments revealed Rf values for "direct" and "indirect" reacting bilirubin azopigments. After hydrolysis of the "direct reacting" azopigment, a substance with the chromatographic behavior of glucuronic acid was obtained and the azopigment had been converted to "indirect reacting."

The following mechanism is consistent with these findings: bilirubin (IRB) plus UDPGA (liver extract) in the presence of liver transferase(s) (microsomes) yields bilirubin glucuronide (DRB).

Liver tissue was obtained at surgery from a patient with constitutional hepatic dysfunction; extract, homogenate and microsomes were immediately prepared from this tissue and from rat liver. The patient's liver extract was capable of increasing the conjugation of bilirubin or orthoaminophenol when incubated with rat liver homogenate or microsomes. The patient's liver homogenate or microsomes did not produce conjugation of these two substances when incubated with an active rat extract. These data suggest that a defect in the transfer of glucuronic acid to bilirubin exists in this disease.

The Ammonia Arterial-Venous Difference in Hepatic Failure, as Influenced by Alterations in Peripheral Circulation

By *Malcolm P. Tyor and Herbert O. Sieker.* Departments of Medicine, Duke University School of Medicine and Veterans Administration Hospital, Durham, North Carolina.

Venous ammonia concentrations have been used extensively to study and manage patients with hepatic failure. A peripheral arterial-venous difference of ammonia has been observed in these patients, who also show disturbances in peripheral circulation.

The ammonia A-V difference in the arm was determined in 15 patients with fluctuating hepatic failure and elevated arterial ammonia concentrations. Serial samples in individual patients were obtained in the same environment from the same vessels. A significant positive A-V difference was observed in 82% of 60 determinations. Despite considerable scatter, the magnitude of the A-V difference could be correlated with the height of arterial concentration. However, repeated observations in individual patients showed (1) no appreciable A-V difference despite arterial concentrations ranging from 165 to 560 μg. %; (2) fluctuating A-V differences, which are unrelated to arterial concentrations, e.g., arterial = 215 and 440 μg. %, A-V = 120 and 65 μg. %, respectively.

Ammonia A-V differences determined serially in 8 cirrhotic patients, following a standard oral ammonium chloride load, showed the same trend, as regards correlation with arterial concentration. However, individual patients showed (1) marked elevations of A-V difference, 350 μg. %, with comparatively little change in venous level; (2) no A-V difference at arterial concentrations of 350 μg. %; (3) fluctuating A-V differences despite similar arterial concentrations, when studied on separate days.

Ammonia and oxygen A-V differences, sampled simultaneously, were compared in 5 patients. A positive correlation was observed and was most apparent from multiple determinations in individual patients, e.g., ammonia = 112, 48, 12 μg. %, oxygen = 6.27, 3.83, 2.03 vol. %, respectively. No consistent trend was observed, when ammonia A-V differences were compared with arterial oxygen saturations and pCO_2.

Ammonia arterial-venous differences in hepatic failure are influenced by arterial ammonia concentration and an altered peripheral circulation. Venous ammonia concentrations are unreliable in hepatic failure.

The Kinetics of Diiodotyrosine Metabolism in Normal Subjects and in Patients with Liver Disease

By *W. R. Ruegamer and R. B. Chodos.* Radioisotope Service, Veterans Administration Hospital, Syracuse, and the Departments of Biochemistry and Medicine, State University of New York, Syracuse.

In order to derive a mathematical expression for the kinetics of diiodotyrosine (DIT) metabolism in man, studies were made of the fate of intravenously administered I^{131}-labeled DIT in normal subjects and in patients with liver disease. Paper chromatographic analysis was employed to determine the distribution of I^{131}-labeled substances in the plasma, saliva, and urine.

During the first 24 hours, all of the administered radioactivity was recovered as DIT, or as iodide. The DIT disappearance curve could be expressed as an exponential equation with two components. The first component was very rapid and probably represented distribution in body fluids. The second component yielded a half-time value of 3.3 hours in normal subjects and 6.0 hours in patients with liver disease. This component probably represented deiodination of DIT.

Whole plasma radioactivity measurements and half-time values indicated not only DIT disappearance but also the rate of I^{131} iodide liberation from DIT and the subsequent redistribution and excretion

of iodide. The rate of deiodination of DIT was such that the whole plasma radioactivity was predominantly I^{131} iodide after 10 hours. Thereafter, the salivary secretion, urinary excretion, and thyroid accumulation of I^{131} was a function of the plasma concentration of I^{131} iodide and the respective metabolic rate constants of these tissues.

The rapidity of DIT disappearance provides additional evidence that the metabolism of DIT in normal subjects and in patients with liver disease is not a limiting factor in the degradation of thyroid hormone.

Peripheral Oxygen Uptake and Lactate Production in Patients with Cirrhosis of the Liver at Rest and During Exercise

By *Walter H. Abelmann, Ernest W. Hancock and Rhett P. Walker.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston. (Aided by a grant from the Life Insurance Medical Research Fund.)

Is increased peripheral (muscle) blood flow in patients with cirrhosis, demonstrated previously, associated with increased peripheral metabolism?

Blood flow was estimated by venous occlusion air plethysmography, and arteriovenous oxygen and lactate differences were measured in the resting and steadily exercising legs of 7 patients with cirrhosis of the liver and of 6 control subjects. Patients with evidence of heart disease, edema, thiamine deficiency or elevated systemic metabolic rate were excluded.

At rest the mean oxygen uptake was 0.50 ml./min./Kg. leg ± 0.092 (S.E.M.) in the control group and 1.07 ml./min./Kg. ± 0.226 in the cirrhotic patients. During exercise the oxygen uptake rose to 1.24 ml./min./Kg. ± 0.333 in the control group and to 2.43 ± 0.435 in the cirrhotic patients. The differences between the means of the two groups are significant ($p = 0.05$) at rest and during exercise. Resting lactate production was 0.06 mg./min./Kg. ± 0.021 in the control group and 0.23 mg./min./Kg. ± 0.035 in the cirrhotic group, and during exercise rose to 0.26 mg./min./Kg. ± 0.091 in the former and to 1.39 mg./min./Kg. ± 0.357 in the latter. The differences between the two groups are significant ($p = 0.01$).

Increased aerobic and anaerobic peripheral metabolism was associated with increased peripheral blood flow, increased arteriovenous differences of oxygen and lactate, or both.

It is concluded that increased peripheral blood flow in patients with cirrhosis may, at least in part, be metabolically justified. Increased peripheral lactate production is a factor in the increased blood lactate levels associated with cirrhosis, which previously have been attributed directly and entirely to hepatic insufficiency.

Nitrogen and Electrolyte Metabolism During Neomycin Administration in Cirrhotic Patients

By *William W. Faloon and Curtis J. Fisher.* Department of Medicine, State University of New York Upstate Medical Center, Syracuse.

The efficacy of oral Neomycin in controlling blood ammonia concentration in hepatic cirrhosis has led to an investigation of its metabolic effects.

Six patients with hepatic cirrhosis and one with malnutrition were fed diets constant in nitrogen, potassium, sodium and calories for 4 to 6 day control periods, 6 to 7 days of Neomycin 12 Gm./day orally or 500 mg. intramuscularly, and 5 to 6 day post-Neomycin periods. In 4 patients receiving oral Neomycin and in one receiving parenteral Neomycin complete balance studies of nitrogen, potassium and sodium were done. In one patient only urine determinations and in another only fecal analyses were done.

Oral Neomycin resulted in increased nitrogen anabolism in two patients and no change in two. Fecal nitrogen excretion was unchanged in one patient, rose by 0.6 to 0.9 Gm. daily in 3, and by 3.0 Gm. in another. In the latter patient, however, nitrogen balance was unchanged. A decrease in urine nitrogen of 4 Gm./day occurred in the patient in whom only urinary analyses were done.

Increased fecal potassium of from 9 to 16 mEq. daily occurred in four of five patients but urinary potassium decreased proportionately and potassium balance varied with nitrogen balance. Fecal sodium increased by 8 to 30 mEq. daily in 3 patients but net sodium retention occurred in two of these and in another patient without fecal sodium change. Parenteral Neomycin had no metabolic effects.

These results indicate that Neomycin has no deleterious effect upon nitrogen metabolism and may enhance nitrogen anabolism. This occurs even in the presence of increased fecal nitrogen and suggests that absorbed nitrogen is more efficiently utilized. Despite increased fecal electrolyte excretion renal retention of these ions appears to compensate adequately when normal electrolyte intake is maintained.

Blood Pressure Levels in Cirrhosis of the Liver

By *Louis J. Vorhaus.* Department of Medicine, New York Hospital-Cornell Medical Center, New York.

Much has been said, but very little written, on the subject of blood pressure in patients ill with cirrhosis of the liver. It is generally felt that cirrhotics have lower blood pressures than otherwise healthy individuals of a comparable age and sex. In this study, observations were recorded on 344 cirrhotics with respect to their blood pressure and the severity

of their disease as judged by clinical and laboratory data. It was noted that, as a group, the cirrhotics studied did not show blood pressure levels that were significantly low, nor was there any observable relationship between the severity of the disease and the level of blood pressure in individual patients, except among those in coma who tended to show hypotensive levels. Serial observations were recorded on 52 patients, followed over several years, some of whom improved while others deteriorated. There were no observable changes in the blood pressures of these patients during the interval they were under observation, regardless of the changes noted in their clinical status. Lastly, cold pressor tests were performed on a group of 36 patients ill with cirrhosis. It was observed that their responses were entirely comparable to those of healthy individuals of comparable age, and a number of patients even exhibited responses characteristic of labile hypertensive subjects. It is concluded that, in this group of patients, blood pressure levels were not abnormal, did not correlate with the extent and severity of the disease, and did not change with time despite changes in the clinical status of the patient.

Immediate Changes in Blood Volume Following Paracentesis

By *Alvin S. Wenneker and George L. Fischer.* Department of Preventive Medicine, Washington University School of Medicine, and the Washington University Medical Service, Veterans Administration Hospital, St. Louis.

The following study was undertaken to determine the changes in blood and plasma volume which occur in the period immediately following paracentesis in Laennec's cirrhosis. Contraction of blood volume has been demonstrated previously 24 hours or later following paracentesis.

Blood volume was determined by the radioactive chromium-tagged red blood cell method. On the following day, an adjusted blood volume was calculated on the basis of remaining radioactivity. Paracentesis was then performed, and blood volume was determined immediately, one-half, one, 2, 3, 6 and 24 hours later. Red cell mass and plasma volume were calculated on the basis of venous hematocrit. For comparison, determinations were performed on both normal and cirrhotic control subjects, not undergoing paracentesis.

A small drop in blood volume, ranging from 3.2 to 8.4%, occurred at the end of the paracentesis in 4 of 7 studies in 6 patients. In all patients studied, the blood volume subsequently rose during the first 6 hours, the maximal increase ranging from 6.3 to 18.7%. The highest average increase was observed at 2 hours and equaled 10.0 ± 2.2% (S.E.). Red cell mass remained relatively constant, and the increase in blood volume was attributable to a change in plasma volume. The maximal rise in plasma volume during the first 6 hours varied from 13.8 to 27.9%.

In the control subjects the blood volume remained essentially constant over this period of time. The maximal increase in blood volume in any one control subject during the first six hours was 3.2%; the greatest average variation was observed at 6 hours and was equal to a contraction of 6.3%, probably attributable to fluid restriction during this period.

The rise in blood volume observed during the period immediately following paracentesis is significant by statistical tests.

MUSCLE

The Effect of Potassium on the Resting Membrane Potential of Skeletal Muscle in the Intact Rat

By *John C. Harvey and Kenneth L. Zierler.* Departments of Environmental Medicine and Medicine, The Johns Hopkins University and Hospital, Baltimore.

It has been held that the resting membrane potential (E_r) in nerve and muscle cells is determined largely by the log of the ratio between the intracellular and extracellular potassium concentrations. It should be possible, within limits, to alter the potassium concentration of extracellular fluid and produce a predictable change in E_r. Resting membrane potentials were measured with KCl-filled glass microelectrodes introduced into single muscle fibers of the gastrocnemius of the intact rat. In 500 measurements in 49 rats E_r was 80.2 ± 7 mV. In 25 rats the initial serum K^+ concentration, measured from jugular blood, varied over a sufficiently wide range to test the hypothesis. E_r varied inversely with log of serum K^+ concentration. Infusion of KCl (0.04 M and 3 M), but not of NaCl, at the bifurcation of the aorta through a catheter inserted into the contralateral femoral artery caused a prompt fall in E_r which was maintained throughout the period of constant injection.

Blood Flow to Skeletal Muscle as Studied by Polarography

By *F. S. Caliva, P. V. Gabel and R. H. Lyons.* Department of Medicine, State University of New York Medical Center, Syracuse.

The technic of polarography has been adapted by the authors to the study of circulation in the intact skeletal muscle of dogs. Teflon-coated platinum electrodes were introduced through the lumina of #20 gauge needles into the muscle. Ample evidence has been accumulated to prove that the oxygen changes, electronically recorded, parallel alterations in arterial blood flow. This method offers a means of studying muscle circulation alone. In addition, it gives information concerning effective or nutritional circulation.

The effects of various drugs on the muscle vasculature have been investigated. The vasodilator action of intravenous epinephrine and norepinephrine have been confirmed. Nembutal and Arlidin were also found to be vasodilators. Blocking of the sympathetics with spinal anesthesia resulted in decreased blood flow.

NEOPLASTIC DISEASE

The Tumor Inhibiting Action of a "Heparinoid" Polyethylenesulfonate (PES)

By *William Regelson*. Roswell Park Memorial Institute, Buffalo, New York.

Polyethylenesulfonate (PES), the sodium salt of a polymer of ethylene sulfonic acid, has been shown by others to have many properties in common with heparin. It produces significantly more lipemia clearing activity for equivalent anticoagulant dose.

The effect of native heparin and several "heparinoid" compounds has been studied in Swiss mice bearing transplanted Ehrlich's 2 or Krebs tumors. Thirty million cells were transplanted to the flank on day 0 and drugs administered subcutaneously on days 1 through 5. Tumors were removed and weighed on day 6.

The median weight of Ehrlich's tumor, in one experiment, was 121 mg. After PES at a dose of 100 mg./Kg./day the median tumor weight was 75 mg. (38% inhibition); after PES 400 mg./Kg./day median tumor weight was 55 mg. (55% inhibition). Krebs tumor showed a response similar to that of Ehrlich's.

These and other data show a highly significant inverse linear correlation between log dose and log tumor weight for Ehrlich's and Krebs tumors when treated with PES. At dose levels in excess of 200 mg./Kg. a substantial death rate from hemorrhage occurs. Weight loss in these experiments has been negligible.

Preliminary results suggest that similar tumor inhibition is seen in subcutaneous lymphocytic tumor L 5178, both sensitive and resistant to amethopterin.

Investigation of the Cytologic and Histochemical Characteristics of Metastatic Tumor Cells

By *B. E. Maney, W. W. Thayer and W. C. Moloney*. Hematology Laboratory (Tufts First and Third Medical Services) Boston City Hospital. (Aided by Grants from the Hematology Research Foundation and the American Cancer Society.)

Metastatic cancer cells in pleural, ascitic and pericardial fluids lend themselves very well to cytologic and histochemical studies. Cells can often be obtained in large amounts, free of stroma and in a relatively "pure" population. Aspirates from metastases to bone marrow or lymph nodes are more difficult to obtain and do not represent as "pure" a malignant cell population.

Cytologically, at times it is difficult to distinguish mesothelial from tumor cells in body fluids and it is often impossible to determine the types or source of metastatic cancer cells from morphologic criteria only. Histochemically, the presence of biochemical substances or evidence of enzyme activity in tumor cells may be of assistance in the following ways: (1) to determine whether a cell is neoplastic or not; (3) to establish the type and source of the metastatic cancer cell; (3) to shed some light on the metabolic and biochemical activities in neoplastic cells.

This preliminary report is concerned with morphologic and histochemical studies carried out on a variety of metastatic tumor cells found in body fluids, or aspirated from bone marrow, lymph nodes and tumor masses. Fixed and unfixed preparations were made on cells resuspended in homologous serum and "pulled" on cover slips. Morphologic studies were carried out on Wright or Giemsa stained material. Histochemical methods included PAS stain, Sudan Black B, peroxidase, pyronin-Y-methyl green and other special technics. Methods for detection, localization and characterization of various enzyme activities including alkaline phosphatases, nucleotidases, nucleases, esterases, lipases and sulfatases were employed in this investigation. Studies on cell morphology and localization of biochemical substances and enzyme activity were documented both in color and black and white photomicrography.

Thus far, metastatic cells from cancer of the breast, lung, ovary, large and small bowel and neuroblastoma have been studied. In addition to morphologic observations, evidence for an enzyme specifically dephosphorylating adenosine-5-phosphate has been demonstrated in metastatic ovarian cancer cells. Marked nonspecific alkaline phospho-

monoesterase has been found in metastatic cancer cells from the breast and lung. In certain cases of metastatic lung cancer, the cells have shown a marked esterase activity.

It is evident from these preliminary results that interesting and useful observations can be obtained from histochemical studies of metastatic tumor cells. Moreover, this investigative approach may lend itself to basic studies on the mechanisms of cellular enzyme activity.

Arteriography in Bone and Soft Tissue Tumors with Special Consideration of the Venous Phase

By *Robert Schobinger von Schowingen and Ru Kan Lin.* Department of Surgery and Diagnostic Radiology, Roswell Park Memorial Institute, Buffalo, New York.

A series of 70 benign and malignant, primary or metastatic bone and soft tissue lesions was evaluated by means of angiography. A rather satisfactory comparative evaluation was possible because of standardization of the technic, i.e., execution of all injections by one person, serial exposures of 12 films of 1 second intervals and with the first film exposed at the end of the injection. Each angiographic series was, for the purpose of better evaluation, divided into an arterial, capillary and venous phase. The conclusions to be drawn from the authors' material are as follows:

(1) a normal arterial phase does not preclude an abnormal pattern during the venous phase; (2) all benign lesions did exhibit a normal arterial phase (hemangiomas are not considered in the present study); (3) the majority of benign lesions also demonstrated a normal venous pattern, but giant cell tumor, osteitis fibrosa cystica, aneurysmal bone cyst and non-osteogenic fibroma evidenced roentgenographic retention of contrast medium during the venous phase. Histologic sections suggest that this phenomenon is independent of the degree of vascularity but influenced by the relative number and location of giant cells within these lesions; (4) the majority of malignant neoplasms did exhibit pathologic arterial patterns of the well-known type associated with puddling of contrast medium during the venous phase and occasional evidence of arteriovenous fistulas. The retention of contrast medium during the venous phase was usually 12 seconds or less; (5) normal arterial patterns were observed in subperiosteal fibrosarcoma (some), osteosclerotic metastases and early reticulum cell sarcoma of bone of still intracortical location. The former 2 lesions also had entirely normal arterial patterns; (6) intense roentgenographic retention of contrast medium during the venous phase (12 seconds or more) was noted in Ewing's sarcoma and reticulum cell sarcoma of bone, including the above early case with a normal arterial pattern.

Hence, these findings tend to support the relationship between reticulo-endothelial elements and Ewing's sarcoma on the one hand and giant cells on the other, if the roentgenographic retention of contrast medium is considered as criterion.

The Clinical Significance of Lactic Dehydrogenase Activity of Effusions

By *Felix Wróblewski.* New York.

It has been suggested that cancer cells originate from normal cells by irreversible injury of respiration and replacement of lost respiration energy by fermentation energy. This hypothesis is consistent with the observation that some experimental and clinical states of malignant neoplasia manifest increased serum lactic dehydrogenase (SLD) activity. It was postulated that body fluids in which cancer cells live freely may have increased lactic dehydrogenase (LD) activity.

Pleural, peritoneal and pericardial effusions of 75 patients with cancerous and noncancerous disease were measured for LD activity, and simultaneously the SLD activity was determined. The LD activity of the effusions was correlated with the cytologic flora of the fluid. In addition, the erythrocyte, leukocyte, protein content, color and specific gravity of the effusions were determined.

It appears that effusions containing malignant cells demonstrable by clinical cytologic tecnics manifest LD activity greater than the LD activity of the serum obtained concomitantly from the same individual. Effusions free of malignant cells have LD activities less than the respective SLD.

The identification cytologically of malignant cells in body fluids is sometimes difficult because of confusion in differentiation of histiocytes, mesothelial, and cancerous cells. The confirmation of cytologic diagnosis of malignant cells in effusions by comparison of LD activity of body fluid and serum would appear to be a useful tool for study and diagnosis.

The Distribution of Triethylene Thiophosphoramide in the Human

By *Harry N. Carlton, Jeanne C. Bateman and Gordon E. Lindenblad.* Cancer Research Laboratory, Garfield Memorial Hospital, Washington, D. C.
(Aided by a grant from the Atomic Energy Commission.)

Much clinical evidence has been amassed indicating that triethylene thiophosphoramide is a useful chemotherapeutic agent in the treatment of cancer. Such data has prompted a study of the metabolism of this drug.

To facilitate this study, homogenates were prepared of tissue obtained at autopsy from patients who had been treated with drug in which some of the carbon atoms had been replaced by isotopic

C-14, and some of the hydrogen atoms by H-3 (tritium). Although most of the tagged drug is rapidly excreted in the urine, a small percentage remains in the tissue. The amount remaining in the tissues is more highly concentrated in some organs than in others, and the tumor is not necessarily the site of greatest concentration.

Chromatographic technics indicate that most of the tagged material moves as the parent compound triethylene thiophosphoramide. Since some of the material moves at a different rate, breakdown of the original molecule, or further combination of it with some other moiety, is indicated. Further investigations are in progress to determine the nature of the material which possesses properties other than those of free triethylene thiophosphoramide.

Clinical Experience with Triethylene Thiophosphoramide in Disseminated Malignant Disease

By *William Mabon Davis and Howard Simpson.* Tumor Service and Cancer Clinic of the Westfield State Sanatorium.

A total of 44 patients with a variety of malignant neoplasms, including lymphoma, leukemia, multiple myeloma, malignant melanoma, and carcinoma have recently been treated with triethylene thiophosphoramide (Thio T.E.P.A.). The drug was given intravenously in 10 mg. doses for an initial course of five injections, followed by weekly doses as indicated. Dosage and schedule were modified individually on the basis of response and evidence of hematologic effects. An initial course of treatment was considered adequate therapy. The total dosage received varied between 50 and 240 mg.

Two patients out of five with active Hodgkin's disease obtained good remissions. Two patients in the terminal phase of their disease made no response; one case obtained equivocal benefit. Two patients with chronic lymphocytic leukemia were improved. Treatment was of no benefit in single cases of lymphosarcoma and reticulum sarcoma, two cases of malignant lymphoma unclassified, and two patients with chronic myelocytic leukemia. Multiple myeloma in four patients was not influenced by treatment. One patient with malignant melanoma showed striking regression of tumor masses; five other patients were failures. Three of eight patients with lung carcinoma were improved subjectively. Two cases of widespread ovarian carcinoma were benefited. Treatment in seven remaining cases of advanced carcinoma and one case of seminoma was unsatisfactory.

No serious toxic effects occurred except in one patient who inadvertently received 100 mg. in lieu of the usual 10 mg. dose, after previous total dosage of 60 mg. A profound leukopenia and thrombocytopenia occurred from which he slowly recovered after six weeks. Definite but unsustained improvement occurred with this hematologic reaction; there was no serious bleeding or any clinical effect from the marrow depression.

In summary, Thio T.E.P.A. is an effective palliative agent in the treatment of certain cases of leukemia and lymphoma and in certain forms of metastatic carcinoma.

Fluoxymesterone in the Treatment of Advanced Breast Cancer

By *B. J. Kennedy.* Minneapolis

Fluoxymesterone (9a-fluoro-11B-hydroxy-17a-methyltestosterone) (Halotestin) has been administered to 33 consecutive patients with advanced breast cancer. It is an *oral* androgenic-anabolic hormone approximately five times as potent as methyl testosterone in the human. Therefore, it can be administered in doses comparable to that of intramuscular preparations of testosterone propionate. A dose of 10 mg. every day was employed.

Objective regressions of metastatic breast cancer occurred in 16 of 30 patients. Subjective improvement occurred in 17 patients. The quality of improvement was similar to that previously recorded with testosterone propionate. The incidence of objective improvement is at least comparable to that of testosterone propionate. Metabolic studies of calcium excretion support the clinical improvements noted. There was a decrease of urinary calcium excretion, a decrease of hypercalcemia, and an increase in serum alkaline phosphatase.

Observations of the side effects of fluoxymesterone reveal masculinizing changes. The degree of hirsutism, acne, and increased libido was less than with testosterone propionate, though hoarseness was similar. An increase in erythropoiesis occurred. Induced hypercalcemia was observed as with other androgenic agents.

It would appear that fluoxymesterone is a potent androgenic-anabolic hormone that is an effective agent in the treatment of advanced breast cancer and may be substituted for testosterone propionate.

Polyethylene-Imines in the Treatment of Solid Neoplastic Disease

By *M. J. Brennan and G. Betanzos.* Oncology Division, Henry Ford Hospital, Detroit.

Forty-three patients suffering with a wide variety of solid tissue malignancies have been given 48 courses of therapy with polyethylene-imines in the past 30 months. An additional 5 patients have been treated for granulocytic leukemia. Of those with solid tissue neoplasia, 35 have been treated with triethylene thiophosphoramide, 3 with triethylene phosphoramide and 10 with triethylene melamine.

An intensity factor calculated from the equation, I equals milligrams per kilogram per day times

1,000, has been related to the results and complications of therapy. Duration of disease and pretreatment and clinical status have also been related to results.

The differences in efficacy of these agents in clinical medicine and their efficacy in experimental neoplasia will be discussed. Though treatment has been carried to a level of intensity productive of hematopoietic depression of the maximum tolerable degree, the phosphoramides have failed to yield significant palliative results in this group of patients in whom the neoplasia had the following origins: melanoma, 7; nasopharynx, 3; larynx, 1; lung, 7; breast, 2; cholangio-carcinoma, 1; hypernephroma, 2; pancreas, 1; colon, 4; ovary, 1; uterus, 1; bladder, 1; soft tissue, sarcoma, 5; and undetermined, 7.

Studies on the Mechanism of Altered Calcium Metabolism Induced by a Metabolic Antagonist Diazo-oxo-norleucine

By *W. P. Laird Myers.* Memorial Center, New York.

6-diazo-5-oxo-L-norleucine (DON), has been found to reverse hypercalcemia and hypercalcuria due to bone metastases in 6 of 15 patients. Since DON inhibits growth of experimental tumors and since evidence suggests that correction of calcium abnormalities indicates decreased osteolysis secondary to a reduced rate of tumor growth, the mechanism of DON-induced effects was ascribed to a probable tumor-inhibiting action of the compound. However, none of the patients so treated showed evidence of bone healing roentgenographically, and objective signs of regression of soft tissue tumor were sparse. Accordingly, the following studies were done to evaluate the possibility that DON was affecting calcium metabolism without affecting tumor growth:

(a) Altered fecal calcium excretion was excluded by a balance study which revealed no rise in fecal calcium during DON administration despite a fivefold decrease in urinary calcium; (b) chelation of calcium by DON could not be demonstrated by in vitro studies. A balance study in a normocalcemic patient with hypercalcuria revealed no hypocalcemia or change in calcium balance with DON. These data are evidence against a non-specific effect on calcium metabolism; (c) Altered renal mechanisms could not be invoked as a cause for the decreases in serum calcium: on DON, GFR and RBF remained unchanged in a normocalcemic patient, and calcium diureses were not observed in hypercalcemic patients; (d) DON caused no significant changes in sodium or potassium balances and no disturbances in serum electrolytes, indicating that the observed changes were not mediated through alterations in other ions.

Although DON inhibition of a hypothetical bone-resorbing substance secreted by the tumor is a possibility that cannot be excluded, the data tend to support the conclusion that DON affects calcium metabolism by a carcinostatic action. This viewpoint is strengthened by an apparent specificity of tumor response, since DON does not produce these changes in all patients.

NERVOUS SYSTEM

Studies in Human Cerebral Hemisphere Function: Impairment of Highest Integrative Functions After Occipital Lobectomy

By *Loring F. Chapman, William N. Thetford, Thomas C. Guthrie, Louis Berlin and Harold G. Wolff.* Study Program in Human Health and the Ecology of Man and the Departments of Medicine (Neurology) and Psychiatry, The New York Hospital and Cornell Medical Center, New York.

Twelve subjects who had undergone occipital lobectomy for arteriovenous anomalies were studied in the laboratory and in their natural setting as they sought to resume their lives. They were studied by means of (1) extensive interviews with regard to life patterns before and after tissue loss; (2) intensive survey of behavior, including data collected from members of their families, neighbors, friends, and employers, relating to their biographical and current environmental settings, their behavior, mentation, attitudes, thoughts, mood, and bodily reactions, as well as their over-all (interpersonal, social, and vocational) adjustment capacity before and one to five years following cerebral hemisphere tissue loss; (3) a large group of quantitative behavioral indicators (Wechsler Bellevue Scale, Halstead Battery, Rorschach Ink Blots, Conditioned Reactions, Reactions to Failure, and others). It was found that these subjects, in addition to having defects in their visual fields, were impaired in their highest integrative functions. The degree of impairment was comparable to that observed in subjects with loss of tissue of similar mass elsewhere in the cerebral hemispheres. After large (60–90 Gm.) occipital lobectomy, many of the changes that were also observed in subjects who had undergone incomplete (60–90 Gm.) frontal lobe resections were noted, including altered reactions to painful stimuli, hyperactivity, cyclic fluctuations of mood and activity, "irritability," ready fatigability, freedom from preoccupation with personal anxieties and conflicts, defects in planning and initiative, inability to sustain purposive effort for more than brief periods, major memory deficits and a slower rate of accomplishing tasks. Subjects with occipital lobe loss performed relatively less well on assay pro-

cedures requiring rapid visual synthesis, visual imagery, or visual perception in the face of simultaneous and irrelevant visual stimuli than did subjects with equivalent mass of tissue loss from the frontal lobes.

Acquired Tolerance to Nervous Tissue in Rats

By *Philip Y. Paterson.* Department of Microbiology, University of Virginia, School of Medicine, Charlottesville.

The injection of mammalian nervous tissue in adjuvant into rats and other laboratory animals is known to induce striking paralysis and complement-fixing (CF) anti-brain antibodies. This condition is called experimental encephalomyelitis and is presumed to be "allergic" in nature. It appears similar to or identical with post-rabies vaccination paralysis and post-infectious encephalitis of humans.

Work in this laboratory indicates that rats exposed neonatally to nervous tissue and subsequently challenged with nervous tissue-adjuvant have limited capacity to develop encephalopathy but little, if any, impairment of CF anti-brain antibody production. Each of 32 new-born Wistar stock rats received guinea pig spinal cord IP 4 to 25 hours after birth. Controls consisted of 31 litter mates either similarly injected with guinea pig kidney tissue or left uninjected. When 9 to 10 weeks old, all rats were challenged ID with guinea pig nervous tissue in adjuvant. As expected, from other work in this laboratory, 22 of the 31 control rats (71%) showed classical encephalomyelitis following challenge. In contrast, only 6 of the 32 rats (19%) exposed neonatally to nervous tissue showed clinical encephalopathy; the remaining 26 animals appeared perfectly well. Serologic studies revealed little, if any, suppression of CF anti-brain antibody production following challenge in rats exposed neonatally to nervous tissue when compared to control animals. Fourteen of 29 rats (48%) injected neonatally with nervous tissue had CF antibody titers (1:4 to 1:256); 15 of 29 controls (52%) had CF antibody titers (1:16 to 1:512). The data indicate that rats can acquire tolerance to the paralytogenic activity of heterologous nervous tissue and suggest that the mechanisms for the development of paralysis and for the production of CF anti-brain antibodies represent independent events.

Effect of Sound on Survival of White Rats Subjected to Hypoxia

By *Andrew Kerr, Jr. and A. Foster Sanders.* V. A. Hospital, Syracuse; Department of Medicine, State University of New York College of Medicine in Syracuse; and Department of Medicine, Louisiana State University School of Medicine, New Orleans.

This study measured the effect of a sensory modality upon the survival time of white rats in a lethal situation. The modality chosen was sound; the lethal situation was hypoxia. Sound was produced by ringing a household door bell attached to the lid of a metal garbage can. Rats were placed in the can and submitted to 2 minutes of bell ringing. The sound was of sufficient intensity, approximately 90 decibels, to incite convulsive seizures in one third of the animals. Hypoxia was produced in a low pressure chamber by reducing air pressure to 187 mm. Hg. Factors known to affect response to sound and hypoxia were equalized in each experimental group.

Seventy rats were exposed to sound 5 to 7 times at 24–48 hour intervals and 48 hours after the last exposure were tested for survival. 103 rats were exposed once to sound to determine susceptibility to seizures and 30 days later tested for survival. 38 were not submitted to sound before testing for survival.

In one strain ($N = 146$) the median survival time (23.5 minutes) for animals repeatedly submitted to sound was significantly lower ($x^2 = 6.43$, $P < 0.05$) compared to animals exposed once to sound (median = 33.5 minutes). The survival time for animals exposed once to sound did not differ significantly from that for animals not exposed. In another strain ($N = 65$), less tolerant to hypoxia, a significant ($t = 2.14$, $P < 0.05$) reduction of mean survival time (8.62 minutes) was noted for animals repeatedly exposed to sound compared to animals exposed once (12.75 minutes). The survival times for animals with seizures were not significantly different from comparable animals without seizures.

Repeated exposure to a sensory modality—sound—adversely affects survival of white rats to the lethal situation of hypoxia.

The Clinical Signficance of Alterations in Lactic Dehydrogenase Activity of Spinal Fluid

By *Felix Wróblewski, Barry Decker and Rita Wróblewski.* Sloan-Kettering Institute, New York.

Lactic dehydrogenase (LD) activity of spinal fluid obtained from individuals without central nervous system (CNS) disease ranges from 10 to 40 units per ml. This contrasts with the LD activity of serum from normal individuals in whom the serum LD activity ranges from 200 to 680 units/ml. No relationship between serum LD and spinal fluid LD activity has been noted, and each varies independently of the other. The LD activity of spinal fluid bears no relationship to the total protein, serologic reaction for syphilis, leukocyte count, xanthochromia, chemistries, and initial pressure of the fluid.

Observations in vitro and in vivo of malignant neoplasia suggest that the presence of malignant

cells imparts increased LD activity to the medium with which the neoplastic tissue is in contact. The study of spinal fluids from 125 individuals indicates that increased spinal fluid LD activity (65–290 units/ml.) is present when the CNS is involved by metastatic carcinoma, lymphoma and leukemia. Acute meningitis (60–480 units/ml.) and intracerebral hemorrhage (45–240 units/ml.) are also associated with increased spinal fluid LD activity. In most other instances of central nervous system disease, the spinal fluid LD activity is the same (less than 40 units/ml.) as that observed in the spinal fluid in individuals without CNS disease. The mechanisms for increased spinal fluid LD activity in metastatic neoplasia of the CNS, acute meningitis and intracerebral hemorrhage appear to be different and distinct.

Alterations of spinal fluid LD activity appears to be helpful in the differential diagnosis of CNS disease when the enzyme changes are related to the clinical setting.

Changes in Body Composition During the Course of Acute Anterior Poliomyelitis

By *Alexander P. Remenchik, James A. Schoenberger and Josephine M. Dyniewicz.* (Aided by grants from the National Foundation for Infantile Paralysis, United States Public Health Service and the Committee on Research of the Council on Pharmacy and Chemistry of the American Medical Association.)

This study was initiated to ascertain the types of changes that occur in body composition during the course of acute anterior poliomyelitis. One to four serial simultaneous measurements of total exchangeable potassium (K_e), antipyrine space (AS) and radiosulfate space (RSS) were made in 44 patients suffering from acute anterior poliomyelitis. From this data were calculated intracellular water (ICW), lean body mass (LBM), exchangeable potassium/Kg. of lean body mass (K/LBM), and concentration of potassium/L. of intracellular water ($^{[K]}$ICW).

K_e decreased during the course of the illness, and the amount of decrease correlated with the clinical estimate of the extent of muscle wasting. Early in the illness K/LBM was greater (67.5 mEq./Kg.) than normal (60.5 mEq./Kg.) in males, but less (53.4 mEq./Kg.) than normal (60.5 mEq./Kg.) in females; during the course of the illness K/LBM decreased to values less than normal in males and remained depressed in females. Similar changes were noted in the initial values for $^{[K]}$ICW initially, but this parameter approached the normal value during the course of the illness. Most of the patients showed an absolute increase in the AS early in the course of the illness, despite restriction of fluids (<1500 cc./24 hrs) in the presence of a high environmental temperature and elevated body temperatures, with a marked distortion of RSS/ICW from normal values of .33 to values as high as 2.9. This distortion persisted during the course of the illness even though the absolute value of the AS decreased.

These observations of endogenous overhydration indicate that acute anterior poliomyelitis produces a compositional defect that resembles, in part, the "syndrome of depletion" that has been described for various chronic diseases, and suggests the need for re-examining current concepts of nutritional therapy of acute infectious diseases.

RESEARCH METHODS

Evaluation of a Simple Method for Quantitating Lipoprotein Distribution

By *David F. Brown, Robert B. McGandy, Paul F. Formel and Joseph T. Doyle.* Cardiovascular Health Center and the Department of Medicine, Albany Medical College, Albany, New York.

The blood cholesterol is transported mainly in combination with alpha and beta globulins. Disproportionately large amounts of beta lipoprotein-cholesterol, with a reciprocal diminution in alpha lipoprotein-cholesterol, have been reported in ischemic heart disease. This abnormality may reflect a derangement of lipid metabolism associated with atherosclerosis.

Durrum et al. have recently compared lipoprotein-cholesterol distribution with that of lipoprotein-lipid and found that the distribution of these substances between alpha and beta lipoprotein closely parallels one another. In Durrum's series, cholesterol was measured after elution from paper strips on which the lipoproteins had been separated by electrophoresis. The lipoprotein-lipid, also separated by electrophoresis, was measured in terms of its capacity to bind the dye sudan II (oil red O).

In view of the apparent parallelism between lipid and cholesterol distribution, it would seem that measurement of either would have the same significance. As Durrum's method for lipid determination appeared simple and potentially applicable to large scale studies, it was decided to check its accuracy and reproducibility on a group of 50 adults.

By a modification of Cohn's Method X, separation of fractions I, II and III (containing beta lipoprotein) and fractions IV and V (containing alpha lipoprotein) was effected on 1 ml. samples of serum from the same 50 subjects. The cholesterol content of these fractions was measured. Duplicate deter-

minations of lipid distribution were carried out and results found to be highly reproducible when the dye sudan II was used. Durrum's results were confirmed by demonstrating that lipoprotein-lipid and lipoprotein-cholesterol distribution closely agreed over a wide range of values.

It is thus felt that lipoprotein-lipid, when expressed in terms of capacity to bind the dye sudan II, is as useful a measurement as lipoprotein-cholesterol. In view of the accuracy of the method and the ease with which it can be carried out, it is felt to be most satisfactory for large scale studies.

Evaluation and Comparison of the Ethylenediamine and the Potassium Ferricyanide Methods for the Quantitative Determination of Epinephrine and Norepinephrine

By *M. Sheref Zileli, Fritz W. Reutter, James T. Hamlin and Dale G. Friend.* Peter Bent Brigham Hospital, Boston.

This study was undertaken to determine which of the hydroxyphenol compounds would interfere with the fluorometric determination of the catechol amines in plasma. All compounds were tested by both a modification of the ethylenediamine reaction of Weil-Malherbe and Bone and the $K_3Fe(CN)_6$ reaction of von Euler. Fluorescence was measured with an Aminco-Bowman spectrophotofluorometer.

Epinephrine, norepinephrine, isoprenaline, dopa, dopamine, phenol, catechol, pyrogallol, cresol, phloroglucinol, quinol, resacetophenone and serotonin form fluorescent condensation products with ethylenediamine. Of these, only epinephrine, norepinephrine, isoprenaline, dopa, dopamine, phenol, catechol, pyrogallol and resacetophenone are adsorbed from plasma by Al_2O_3, the latter showing very restricted adsorption.

When the entire series was repeated using the $K_3Fe(CN)_6$ reaction, only epinephrine, norepinephrine and isoprenaline formed fluorescent compounds. The $K_3Fe(CN)_6$ method proved to be more specific but less sensitive than the ethylenediamine method.

Cathechol, phenol and resacetophenone have fluorescent properties similar to norepinephrine and will interfere with its determination. On the other hand, isoprenaline, dopa, dopamine and pyrogallol give fluorescent peaks which interfere with the epinephrine determination. In the $K_3Fe(CN)_6$ method, isoprenaline interferes with both epinephrine and norepinephrine.

The adsorption properties of Al_2O_3 are not specific for dihydroxyphenol compounds as has been reported. These studies demonstrate that certain mono- and trihydroxy compounds are adsorbed and that not all dihydroxy compounds are adsorbed. Adsorption is apparently influenced by the position of the hydroxyl groups on the benzene ring.

Plasma catechol amine studies using the ethyelenediamine method in 20 uremic patients showed markedly increased levels of epinephrine and norepinephrine. Studies are now in progress to determine whether these high levels are caused by an actual increase of epinephrine and norepinephrine or by interference by substances retained or produced by the uremic patient.

Enzymic Activities Altered by Other Enzymes: Possible Clinical Significance

By *Anwar A. Hakim.* Department of Medical Research, National Children's Cardiac Hospital, and Department of Microbiology, University of Miami, Miami.

The action of specific enzymes as determined by in vitro studies, cannot be applied to in vivo activity without careful consideration of the influences of other enzymes or metabolic pool ingredients which are present in the same tissues under conditions existing at the time of observation.

When studied by paper electrophoresis, pepsin is seen to digest DNase (100%) or RNase (100%). After autodigestion of pepsin, similar results are observed using either residual pepsin (DNase, 90%; RNase 88%) or the dialyzing breakdown products (DNase 26%; RNase 67%). Neither acid nor alkaline PHase destroys DNase or RNase. Both dialysate and dialyzed products obtained after interaction of acid PHase and pepsin retain proteolytic activity (dialysate, DNase 29%; RNase 75%: dialyzed product, DNase 88%; Rnase 85%). Observations with dialysate and dialyzed products resulting from action of pepsin and alkaline PHase behave differently (dialysate, DNase 3%; RNase 24%: dialyzed product, DNase 6%; RNase 15%).

These findings indicate that measurement of the activity of an enzyme does not reflect the true quantity of the specific enzyme investigated. The results obtained by such measurement rather represent the total of enzymic activities of each of the enzymes individually, plus the mutual effects of all other enzymes present. Differences in proteolytic activity result from pepsin degradation, when produced by autodigestion, by action of acid PHase, or by alkaline PHase. Thus, active polypeptides liberated are capable of splitting specific interpeptide linkages. These observations assist in the understanding of the relationship between structure and enzymic activity of pepsin.

Because of possible enzymic interrelationships similar to the present observations, although enzyme activities may be useful as clinical aids in the diagnosis of certain diseases, they cannot be used to explain the underlying pathologic mechanisms unless the influences of other enzymes present are evaluated concurrently.

RESPIRATORY SYSTEM

Modifications of the Continuous Cycling Method for Recording Pulmonary Compliance

By *William J. Kuzman, Herman F. Froeb and Hurley L. Motley.* Cardio-Respiratory Laboratory, University of Southern California School of Medicine, Los Angeles.

Measurements of pulmonary compliance were determined by the continuous cycling method, using previously unreported modifications of the method designed to speed up the procedure. The recording device consisted of a Benedict-Roth metabolism apparatus equipped with a helipot for volume changes and a blower without valves to reduce breathing resistance. Oxygen was added to the system and the CO_2 was absorbed. Volume and pressure changes were recorded on a Dumont Cathode Ray Oscilloscope through a compliance control apparatus employing a capacity transducer in a radio-frequency circuit. The loop was interrupted by means of a blanking generator which allowed time measurements at 5-10-15-20 cps intervals. In addition, the no-flow points were determined by a flow-sensitive zero pressure device which produced blips on the oscilloscope at the end of expiration and inspiration. A balloon, as described by Crane, was used to measure the intraesophageal pressure at its midposition. The loop was then photographed by a Polaroid Land Camera. Patients were studied in the sitting position during spontaneous quiet breathing.

This modified procedure was used in a group of 15 normal individuals and gave a mean compliance value of 166 ml./cm. H_2O (range 91–304). The reproducibility of the method was determined in a group of 25 normals and patients with pulmonary disease and found to be ± 11.8 ml./cm. H_2O. This method of recording simplified the calculation of compliance, elastic resistance and the work of breathing. Pulmonary compliance determinations including calculations could be obtained readily in 30 minutes.

The compliance values obtained by this method correlate well with those obtained by the interrupted breathing method. Studies were performed in 45 patients with a variety of cardiorespiratory disturbances. A positive correlation was found to exist between the vital capacity and pulmonary compliance. This improved method for determining pulmonary compliance permits speed and ease of determination without loss of accuracy.

Mathematical Analysis of the Role of Contact Time in Alveolo-Capillary Oxygen Exchange in a Fixed Pulmonary Resistance System

By *Kenneth M. Moser, Sol Katz, Georges F. McCormick and Peter C. Luchsinger.* Cardio-pulmonary Function Laboratory, D. C. General Hospital, and Departments of Medicine, George Washington and Georgetown University Medical Schools, Washington, D. C.

Reduction in the cross-sectional area of the pulmonary capillary bed can result in pulmonary hypertension, increased right ventricular work and impaired diffusion of oxygen from alveolus to capillary blood. These abnormalities are manifestations of the high, fixed level of pulmonary vascular resistance imposed by capillary restriction.

The impariment of oxygen diffusion is a consequence of the mandatory acceleration of blood flow through a restricted vascular bed. It indicates that flow has become too rapid to allow enough time for adequate equilibration of oxygen tension between alveolus and capillary blood. This reduction in contact time is evidenced by an abnormally wide diffusion component of the alveolo-arterial (A-a) oxygen gradient. In these fixed resistance systems, the time of contact should bear an inverse relationship to the quantity of blood which must be accommodated in the capillary bed per unit time. Previous studies have shown that the diffusion component of the A-a gradient does vary inversely with the pulmonary blood flow. Time of contact, however, has been inferred from such changes in the A-a gradient, not calculated per se.

Therefore, patients with diffusion insufficiency for oxygen at rest were studied by combined cardiopulmonary technics before and after lowering of the cardiac output with hexamethonium. With data so obtained, the Bohr integration procedure was employed to calculate values of contact time at rest and during ganglionic blockade. In each instance it was found that the time of contact bore an inverse relationship to the pulmonary blood flow, contact time lengthening as flow decreased.

This mathematical relationship defines more clearly the central role of contact time in diffusion insufficiency. Furthermore, it provides a method for more precise calculation of the relationship between blood flow, contact time and the other factors which may affect alveolo-capillary oxygen transfer.

Lung Compliance and Resistance in Pulmonary Edema

By *J. T. Sharp, I. L. Bunnell, G. T. Griffith and D. G. Greene.* Department of Medicine, Buffalo General Hospital and the University of Buffalo School of Medicine, Buffalo.

Lung compliance and resistance were measured in 6 patients in acute pulmonary edema. Simultaneous tracings of air flow, tidal volume and esophageal pressure were recorded as described by Mead and Whittenberger.

While in acute pulmonary edema, compliance values ranged from .019 to .057 L./cm. H_2O, with an average of .035 L./cm. H_2O. These values are generally below those reported by others in patients

with pulmonary congestion. The 6 patients were restudied several days after recovery from acute pulmonary edema, at which time the compliance values ranged from .039 to .213 L./cm. H_2O and averaged .094 L./cm. H_2O. In most patients pulmonary congestion was still present clinically at the time of the second study.

Lung resistance was increased in all patients, falling in the range reported by others in asthma and emphysema. Inspiratory resistance ranged from 5.0 to 12.7 cm. H_2O/L./sec., averaging 9.9 cm. H_2O/L./sec.; expiratory resistance varied from 6.4 to 18 cm. H_2O/L./sec. and averaged 13.2 cm. H_2O/L./sec.

A high level of resistance frequently occurred within the first .1 to .2 second of inspiration. This early inspiratory "peak" resistance varied from 9.1 to 31.5 cm. H_2O/L./sec. and averaged 18.8 cm. H_2O/L./sec. Though the reason for this high resistance at the beginning of inspiration is not clear, it is thought that it may represent an elevated critical opening pressure of terminal lung units.

The Effect of Heart Failure on Pulmonary Function in Patients with Pre-existing Lung Disease

By *Claire Morrison, J. B. Hickam and H. O. Sieker.* Department of Medicine, Duke University School of Medicine, Durham, North Carolina.

It is a familiar clinical observation that cardiac and pulmonary decompensation often occur together in patients with chronic lung disease, particularly pulmonary emphysema and fibrosis. It is generally accepted that pulmonary decompensation may be precipitated by a respiratory infection and that the resultant anoxia and hypercapnia can lead to cardiac failure. However, observation of these patients suggests that in some instances acute pulmonary decompensation may be precipitated by congestive failure in persons with pre-existing lung disease. To investigate this possibility further, observations have been made on the effect of congestive failure on pulmonary function in patients with and without pre-existing lung disease.

Serial studies were conducted during heart failure without apparent infection and during recovery in 7 patients with pulmonary emphysema and fibrosis. Measurements were made of the lung volumes and intrapulmonary gas mixing by an open-circuit helium method, and of arterial blood gases by conventional procedures. Similar measurements were made on 12 patients with congestive heart failure unrelated to lung disease.

During failure, the patients with pre-existing lung disease showed, in comparison with findings after compensation, marked hypoxia and hypercapnia; increase in FRC (by as much as 1500 ml.); and further impairment of intrapulmonary gas mixing. By contrast, the patients with simple heart failure had moderate hypoxia and hypocapnia, reduction in FRC, and no impairment of intrapulmonary mixing. As measured in a few subjects in each group, pulmonary compliances were not very different. Six normal subjects in whom pulmonary congestion was produced by submersion in a pool had findings similar to the patients with simple failure.

It appears that heart failure in certain patients with emphysema can precipitate pulmonary decompensations by increasing bronchial obstruction and impairing gas mixing in the lungs. Such patients may give the false clinical impression that acute pulmonary decompensation has been the primary event.

The Effects of Histamine and Serotonin on Pulmonary Arterial and Venous Resistances

By *Robert P. Gilbert, Hiroshi Kuida, Lerner B. Hinshaw and Maurice B. Visscher.* Department of Physiology, University of Minnesota, Minneapolis.

It has been shown in the intact dog and in the isolated perfused dog lung that endotoxin causes an increase in pulmonary vascular resistance. A considerable proportion of this increase is owing to venous constriction, since there is a rise of pulmonary artery wedge (PW) pressure and of small vein (PSV) pressure without a change in left atrial (LA) pressure. The isolated lung grows heavier and may become markedly edematous (Kuida et al.). The potent vascular effects of histamine and serotonin, their importance in anaphylaxis, and the many similarities between anaphylaxis and endotoxin shock suggested a re-examination of the effects of these agents on the pulmonary circulation.

Pulmonary artery (PA), LA and PW or PSV pressures were measured in the isolated lungs of 8 dogs during perfusion with blood at a measured flow. Changes in weight were recorded continuously. Since the flow was kept constant, arterial resistance was estimated from the PA to PW gradient and venous resistance from the PW to LA gradient. Both agents caused an increase in over-all resistance. Histamine usually caused the weight to rise, while serotonin usually caused the weight to fall. A gain in weight was associated with a proportionately greater rise of the venous resistance, while a fall in weight was associated with a proportionately greater rise of the arterial resistance.

Since both histamine and endotoxin cause dynamic changes which can explain vascular pooling and edema formation, it is not necessary to attribute their action to a *direct* effect on capillary permeability. Also, these results support the view that histamine-like substances may play an important part in the pathogenesis of endotoxin shock.

Effects of Altered Dynamics of the Lesser Circulation on the Pulmonary Diffusing Capacity

By *J. Howland Auchincloss, Jr., Robert Gilbert and Robert Eich.* Department of Medicine, State University of New York, Upstate Medical Center, Syracuse.

The increased pulmonary blood flow in patients with uncomplicated defects of the cardiac septa might be expected to cause an expansion of the pulmonary capillary diffusing surface. This possibility was investigated in 14 patients with left to right intracardiac shunts, and the results were compared with those obtained in 13 patients with valvular heart disease and 19 normal subjects. The carbon monoxide single breath diffusing capacity was used because of its excellent reproducibility in resting seated subjects. Pulmonary blood flow and pulmonary artery pressure were measured in 13 of the patients with shunts and in 5 of those with valvular heart disease.

From the normal series a regression formula with its standard deviation was developed, and only results more than 2 S. D. from predicted were considered abnormal. This formula was: D_{CO} = 37.4 × Body height in meters − 36.5 ± 3.25 cc./min./mm. Hg.

Abnormally high values for D_{CO} were found in 9 of the shunt cases, whereas all of the valvular patients had normal or reduced values. The greatest increases (43 to 79% above predicted) were found in 4 male subjects with interatrial septal defects, systolic pulmonary artery pressures of 35 mm. or less, and pulmonary blood flows approximately 3–5 times the normal resting value. Patients with left to right shunts and pulmonary hypertension had values of D_{CO} which were only slightly elevated or normal, or, in one case with shunt reversal during exercise, reduced.

It is concluded that sustained hyperemia in cases of left to right shunt is usually associated with a measurable increase in the area of the diffusing surface. This effect may be lost with the occurrence of vascular complications and is not seen at any stage in the passive congestion caused by valvular heart disease.

Carbon Dioxide-Induced Dyspnea in Normal Subjects and Patients with Chronic Pulmonary Disease

By *P. Franklin Mullinax, Jr. and John L. Patterson Jr.* Department of Medicine, Medical College of Virginia and Research Department School of Aviation Medicine, N.A.S., Pensacola, Florida.

The dyspnea commonly evoked by inhalation of 7% CO_2 in air was studied in 2 groups: (1) Arterial pH, CO_2 content, calculated pCO_2 and the ratio minute volume (Mv) × 100/maximum breathing capacity (MBC) were correlated with the onset of symptoms in 7 normal subjects. (2) The work of breathing during CO_2 inhalation was studied in 5 normals, 3 asthmatic and 6 patients with emphysema. Analysis of the elastic work of breathing reveals that the area on the pressure-volume diagram commonly represented as the elastic work of inspiration done on the lung has no definite relation to this area. However, if certain assumptions are made, this area can be used in the approximation of net elastic work.

In the 5 normal subjects (group 1) experiencing dyspnea, mean pH, pCO_2 and the ratio MV × 100/MBC changed from control values of 7.429 pH units, 38.2 mm. Hg and 5.33% to 7.347 pH units, 48.6 mm. Hg and 12.3% at the onset of dyspnea.

Two of the 6 patients with emphysema experienced breathlessness. Other experiments were terminated because of objective respiratory distress. A typical comment was: "I wasn't breathless but I was tired." In the emphysema group, control ventilation was 8.45 L./min. (MV × 100/MBC, 41.9%) and at the onset of symptoms or termination of the experiment 15.3 L./min. (MV × 100/MBC, 74.0%). Diminished ventilatory response to CO_2 in these patients largely explains the data for power (Kg.M./min.): normals, 4.45; asthmatics, 3.75 and patients with emphysema, 2.87.

These data and the previous observation of CO_2-induced dyspnea in a respirator patient with complete respiratory paralysis are consistent with the view that dyspnea is the cortical representation of unremoved respiratory stimuli, sensed by the respiratory center. Further, they suggest changes in the sensitivity of the center to be responsible for not only subconscious responses in ventilation but also the conscious perception of dyspnea.

The Oxygen Cost of Breathing in Dyspneic Subjects as Studied in Normal Pregnant Women

By *Richard A. Bader, Mortimer E. Bader and David J. Rose.* The Mount Sinai Hospital, New York.

Considerable attention has recently been given to the possible role of increased work of breathing in the pathogenesis of dyspnea, from the standpoint of both mechanical work done and total energy required. Cournand et al. demonstrated marked increase in the oxygen cost of breathing in mitral stenosis, pulmonary granulomatosis and emphysema. It seemed appropriate to extend these observations by the study of the dyspnea of pregnant women.

This symptom occurs in 60% of normal pregnant females. The energy required for breathing as measured by the oxygen cost of breathing at rest and during hyperventilation was determined in 12 pregnant women who were dyspneic on exercise and 3 normal nonpregnant women, one of whom was followed serially through subsequent pregnancy. In other pregnant women studied, observations were made in the 7th to 9th months of gestation. In 3 control subjects, ten, twelve and twenty observa-

tions were made in the manner described by Cournand et al.

In 2 of the 3 control subjects, the increment in oxygen consumption in cc./L. increment in ventilation was 1.5 in the range of 5–10 L./min/M², and 4.0 in the range of 20–25 L./min./M² ventilation. The third normal control subject had values for increment in oxygen consumption which were slightly higher. Eleven of the 12 pregnant women had elevated oxygen consumption at rest and a much greater increment in oxygen consumption per liter increment in ventilation. This increment, while variable, was approximately 8.5 cc. O_2/L. in the range studied. Serial observations in one subject prior to and during gestation revealed progressive increase in the oxygen cost of breathing during pregnancy.

The increase in work of breathing may play a role in the pathogenesis of dyspnea in normal pregnant females, as has already been suggested for the dyspnea of pulmonary and cardiac diseases.

Pulmonary Ventilation as an Index of Energy Expenditure

By *Amasa B. Ford and Herman K. Hellerstein.* Department of Medicine, University Hospitals of Cleveland, Cleveland.

A striking linear relationship exists between the energy expenditure of the body and pulmonary ventilation. It has been proposed that ventilation, which can be readily measured under field conditions with currently available respiratory meters, can be used to estimate energy expenditure. Such an estimation would reduce or eliminate laborious gas analyses required by the classic methods of indirect calorimetry.

Twenty-one normal factory workers were observed during a complete typical working day, and an average of seven 5-minute measurements were made of ventilation and energy expenditure on each subject at various levels of activity. The resulting 144 observations have been analyzed for the most useful method of predicting energy expenditure from ventilation alone. The common regression line for the combined data is described by the equation $Y = 0.196X—0.378$, where Y is energy expenditure in Calories per minute and X is pulmonary ventilation in liters per minute. Estimates of energy expenditure made from a single observation of ventilation have a standard error of estimate of 0.328 Cal./min. Multiple measurements of ventilation reduce the error by the factor $1/\sqrt{n}$, where n is the number of measurements. Modern respiratory meters are capable of measuring, integrating, and recording ventilation over periods of hours, so that accuracy can be readily increased in measuring the energy requirements of sustained activities.

The data of the present study fall in the range 1.0–5.0 Cal./min. and represent the level of energy expenditure of workers in light industry. It is reasonable to extend the proposed method to higher levels of energy expenditure, since the regression equation derived from this data is matched by the equation $Y = 0.20X—0.4$ derived by Margaria from laboratory observations over a much wider range of energy expenditure.

The Effect of Treatment on the Ventilatory Response to CO_2 of Patients with Chronic Pulmonary Emphysema

By *Elliott L. Goodman, Richard T. Cathcart and George W. Thomson.* The Jefferson Medical College, Philadelphia.

This study was undertaken in order to determine whether or not the impaired sensitivity of the respiratory center of patients with chronic pulmonary emphysema is permanent.

Inspired 5% CO_2 was used as the stimulus and the ventilatory response evaluated before and after treatment. Eighteen pairs of studies (pre- and post-treatment) were performed on 17 patients. Each study consisted of the measurement of lung volumes and ventilatory ability (maximum breathing capacity—MBC). The patients were studied breathing ambient air. To remove the variation in hypnoxic stimuli, all patients were given 100% O_2 to breathe and then a 95% O_2–5% CO_2 mixture. In each of the 3 steady states ventilation was measured and alveolar and arterial gases were determined. The ventilatory response in this study represents the difference in ventilation between the 100% O_2 period and the 95% O_2–5% CO_2 period.

Prior to treatment all patients had a ventilatory ratio (VR)

$$\left[\frac{\text{increase in ventilation } (O_2 \text{ to } O_2\text{–}CO_2)}{\text{initial ventilation } (O_2)} \times 100\right]$$

that averaged 75; the normal in our laboratory averages 160. Following treatment the average VR remained unchanged; however, 8 patients increased their VR over 10, 2 patients decreased their VR over 10, while the remaining 8 had insignificant changes ($\pm$ 10).

There was no relationship between variations in VR and changes in Pa_{CO_2}, pH, or C_{BHCO_3}. All patients whose MBC increased over 5 L./min. had a significant increase in their VR, while 2 of the 3 patients whose MBC decreased over 5 L./min. had a significant decrease in their VR.

When the MBC was progressively decreased in normal patients by means of increasing constriction in the airway, there was a progressive decrease in their ventilatory response to inspired CO_2. This occurred even though the MBC remained greatly in excess of the ventilatory response with an unobstructed airway.

The ventilatory response to 5% CO_2 can be increased following treatment. It appears to be directly related to an improved MBC and not neces-

sarily related to decreases in pCO_2, unsaturation, serum bicarbonate or hydrogen ion. Although improved, the ventilatory response is still markedly subnormal.

Red Cell Changes in Emphysema

By *Joseph L. Grant, J. Robert Edwards, Alistair MacDonald and George H. Stueck, Jr.* Veterans Administration Hospital, White River Junction, Vermont. (Aided by a grant from the National Heart Institute.)

In emphysema, the rise in hematocrit was found proportionately greater than the increase in oxygen capacity. This gave a 16% decrease in the oxygen capacity:hematocrit ratio, i.e., from .469 in 8 healthy medical students to .394 in 22 emphysema patients ($p = <.01$). Since the change was as great with microhematocrits at 26000 RPM as with Wintrobe hematocrits (3000 RPM), plasma trapping is not considered the explanation. Inactive hemoglobin was searched for by comparison of the gasometrically determined hemoglobin values with those derived from carbon monoxide capacity in 3 cases, and from cyanmethemoglobin conversion in 8. No significant amounts were detected. No abnormal pigment was found by filter paper electrophoresis (types A–H) spectrophotometry, or alkali denaturation for fetal pigment. Red cell water concentration was measured in 24 cases, and rises were observed which gave good correlation with the decrease in oxygen capacity: hematocrit ratio (coefficient of correlation—.95).

It is concluded that in emphysema, increase in red cell water occurs and is responsible for the decrease in the oxygen capacity:hematocrit ratio.

Pulmonary Function in Bronchiectasis

By *Kenneth L. Vosti, Joyce Pearson, George Saxton, Mark Lepper and Harry F. Dowling.* Department of Medicine, University of Illinois College of Medicine, Chicago.

Pulmonary function tests on 23 patients with bronchiectasis included measures of ventilation, distribution of inspired gas, diffusion barrier and venous admixture.

The mean per cent expected vital capacity (VC) was 64 ± 4(SE_m), the mean per cent expected timed vital capacity (1° VC) was 58 ± 3 and the mean per cent expected maximum breathing capacity (MBC) was 45 ± 5. Coefficients of correlation of the percentages of expected VC and 1° VC with MBC were .646 and .648 respectively.

Among 21 patients, only 4 had nitrogen washouts over 2.5% at seven minutes. However, when the patients took a deep breath and expired forcibly three times during the test, peaks of nitrogen concentration were obtained in 16 of 21 patients. These peaks were unaffected by postural drainage and bronchodilators. Similar peaks occurred in some patients with bronchial asthma and emphysema, but not in normals. In asthma, such nitrogen peaks were reduced by bronchodilators, and in emphysema the 7-minute nitrogen concentration almost always exceeded 2.5%.

Intrapulmonary gas exchange studies revealed that half of 16 patients had alveolar-arterial (A-a) Po_2 differences over 10 mm. Hg when breathing low oxygen. Among 18 patients who received 30% oxygen, A-aPo_2 differences were between 41 and 50 in 3 cases, and over 50 in 13.

Interpretations are: The average ventilatory ability of these patients was reduced by one-third. Increased resistance to air flow contributed greatly to this. Maldistribution of inspired gas occurred in three-fourths of patients, and arterial saturation below 95% on breathing room air in two-thirds. Studies during high and low oxygen breathing revealed that half had a significant diffusion barrier, and three-fourths had significant venous admixture. An addition to the standard nitrogen washout tests including maximum inspirations and expirations revealed peaks that apparently represent maldistribution in patients whose 7-minute nitrogen washout value was normal.

Acid-Base Relations between Spinal Fluid and Plasma, with Special Reference to the Control of Ventilation

By *Eugene D. Robin, Robert D. Whaley, Charles H. Crump, Albert G. Bickelmann and David M. Travis.* Department of Medicine, Harvard Medical School and the Medical Clinics of the Peter Bent Brigham Hospital, Boston. (Supported in part by a Grant from the National Heart Institute of the National Institutes of Health, Public Health Service, and in part by a Grant from the Massachusetts Heart Association.)

Previous workers have assumed that the acid-base environment of the respiratory center is more accurately reflected by pH changes in spinal fluid than by pH changes in arterial blood. To test this assumption, simultaneous determinations of plasma and spinal fluid pH, total CO_2 and pCO_2 were performed under various experimental conditions.

In 11 dogs the spinal fluid pH was found to be significantly lower (mean pH 7.32) than the pH of the arterial plasma (mean 7.37) while the pCO_2 of spinal fluid was significantly higher (mean pCO_2 47) than the pCO_2 of the arterial blood (mean 40). Bicarbonate concentration was not significantly different in the two compartments.

The intravenous administration of ammonium chloride or hydrochloric acid produced arterial acidosis and a simultaneous shift of spinal fluid in an alkaline direction. pCO_2 decreased in both compartments. These observations were confirmed by the maintenance of a fixed ventilation and a constant arterial pCO_2. Administration of ammonium chloride

under these circumstances still produced arterial acidosis but no change in spinal fluid pCO_2 or pH.

The intravenous administration of sodium bicarbonate produced a simultaneous arterial alkalosis and a spinal fluid acidosis. There was an increase in pCO_2 of both plasma and spinal fluid. Maintenance of a constant plasma pH by artificial hyperventilation eliminated the paradoxic acid shift of spinal fluid. Under these circumstances spinal fluid pH and pCO_2 remained constant.

These data indicate that the acid-base characteristics of spinal fluid are dependent on two facts: that the spinal fluid compartment is permeable to CO_2 and that changes in the concentration of bicarbonate take place slowly. The acid-base relations of spinal fluid differ from those of extracellular fluid generally and probably do not reflect the acid-base environment of the respiratory center.

A Study of Gastric Secretions in Pulmonary Emphysema

By *Louis Zasly, John M. Rumball and George L. Baum.* Veterans Administration Hospital, Coral Gables, Florida.

Various reports have appeared, referring to the association of chronic peptic ulceration and chronic pulmonary emphysema. Tension, cigarette smoking, and stress are factors that have been thought to influence the association of these apparently dissimilar diseases.

Twelve patients with chronic pulmonary emphysema were studied. Selection was based upon clinical and laboratory determinations, including measurements of pulmonary function. Patients were studied under basal conditions. Volume, pH, and free and total acidity determinations were performed on 20 to 30-minute specimens of gastric juice obtained by continuous suction. Specimens were collected before, during, and following 20 to 30 minutes of inhalation of 5% CO_2. Arterial blood was collected for per cent saturation, pH, and CO_2 content and tension ($PaCO_2$) simultaneously with the gastric juice collections.

Ten patients exhibited a rise in $PaCO_2$ from 4 to 22 mm. Hg, with a mean of 11 mm. Hg. Of these, 7 subjects showed a drop in gastric juice pH from .04 to 2.9 units (mean, 0.607). Increases in volume, varying from 1.5 cc. to 27 cc. occurred in 6 patients (mean, 13.9 cc.). There were increases in free HCl from 2.5 to 36 units in 6 patients (mean, 15 units). There were no changes in $PaCO_2$ in 2 subjects. These exhibited increased gastric volumes and acidity.

These results may indicate a possible relationship between hypercapnia and peptic ulceration in chronic pulmonary emphysema. Further studies are in progress.

The Cytology of Nonmalignant Pleural Fluid

By *Rolf D. Zilversmit, John M. Storer and Theodore H. Spaet.* Department of Hematology, Division of Laboratories, Montefiore Hospital, New York.

The cytology of pleural fluid has been studied in preparations stained with Wright's and Giemsa technics. Preparations consisted of smears from pleural fluid sediment obtained as soon as possible after aspiration. Specimens in each case were from patients with known diagnosis. About 65% of such specimens are suitable for cytologic evaluation.

Cells found in all pleural fluids include erythrocytes, lymphocytes, and "mesothelial" elements. Most also contain variable numbers of neutrophils. Three types of cells, presumably of mesothelial origin, can be recognized. The classification follows that given by Heilmeyer and Wegeman. Type I is a large cell (30–50 microns) with a faintly basophilic, abundantly vacuolated cytoplasm. The vacuoles are small, and their outline blends with the surrounding cytoplasm. The nucleus is approximately 10–15 microns; it is round, eccentrically located, and its outline is regular and clearly demarcated from the surrounding cytoplasm. The nucleus is pale staining, and several nucleoli are typically present. Type II is of similar size, but has cytoplasm free of vacuoles which stains faint pink to violet. The nucleus is comparatively small, is eccentrically located and stains more intensely and uniformly than the nucleus of Type I cells. Nucleoli are usually absent. Cells of type III vary markedly in size and morphology. The diameter ranges from 15 to 55 microns; the cytoplasm is blue-green to intense blue, and no granules or vaculoles are present. The nucleus is 10–15 microns, round, and eccentrically located. It is usually clearly outlined and vesicular in appearance. Nucleoli may be present, but they are fewer in number than those seen in Type I cells. Type III cells are often confused with tumor cells, since multinucleated forms are seen, and clumps of these cells are sometimes present.

In pleural fluid the normal hematogenous elements may undergo a variety of types of distortion, and the presence of fibrin may cause clumping of cells to give a pattern resembling clumps of tumor cells. The distinctive cytologic patterns of many nonmalignant pleural effusions may be of considerable diagnostic value.

The Effect of Progesterone on the Respiration of Patients with Emphysema and Hypercapnia

By *John M. Tyler.* Lemuel Shattuck Hospital, Boston.

It has been shown that progesterone lowers the alveolar pCO_2 of normal men and may be responsible for the lowered pCO_2 in pregnancy. Since retention of CO_2 becomes a problem in decompensated em-

physema, the effect of progesterone was studied in 6 hypercapnic emphysematous patients.

In every instance, progesterone, 50 mg. given intramuscularly daily for one week, caused a lowering of an elevated pCO_2 (mean -10.0 ± 1.5 mm. Hg, $\pm$ S.E., $p < .001$). In each subject, pretreatment controls on two different days checked within 1 mm. Hg (range 46 to 63 mm. Hg). When the drug was discontinued, the pCO_2 increased (mean $+9.4 \pm 2.1$ mm. Hg, $p < .02$). Associated with the fall in pCO_2 as a rise in minute ventilation ($+1.67 \pm .52$ L./min., $p < .05$); alveolar ventilation ($+.90 \pm .17$ L./min., $p < .01$) and in pH ($+.04 \pm .01$, $p < .02$). After treatment there was a prompt reversal; mean decreases were $-1.58 \pm .59$ L./min. ($p < .1$); $-.82 \pm .19$ L./min. ($p < .02$) and $-.03 \pm .01$ ($p < .02$), respectively. During treatment the respiratory rate and the tidal volume were increased in most instances. V_D/V_T was not altered. In three patients $\dot{V}_{O_2}$ rose along with $\dot{V}_E$. In one patient receiving progesterone there was a further rise of $\dot{V}_A$ of 1.2 L./min. when aspirin was added. Simultaneously $\dot{V}_{CO_2}$ increased 46 cc./min. and the pCO_2 fell only 2 mm. Hg, apparently because the efficiency of breathing was so poor that a further rise in ventilation was ineffective in lowering the pCO_2.

Oral anhydrohydroxyprogesterone, 50 mg. q.i.d., was ineffective in 2 patients who subsequently responded to progesterone.

In one subject with clinical deterioration, the pCO_2 rose from 51 to 63 mm. Hg. Without other change in therapy, intramuscular progesterone produced a fall to 45 mm. Hg. Since this level has been maintained with administration of 50 mg. every third day, progesterone may have therapeutic possibilities. Further studies are in progress to determine the minimum effective dose.

RHEUMATIC STATES

Agglutination Tests and Indices of Inflammatory Activity in the Diagnosis and Evaluation of Rheumatoid Arthritis

By *James Crittenden and William J. Kuhns.* Central Blood Bank and the Department of Pathology, University of Pittsburgh Schools of the Health Professions, Pittsburgh.

Sera and/or euglobulin fractions from 77 patients with joint diseases were studied to determine the relative diagnostic importance of sensitized sheep red cells (SSC) and polystyrene latex particles treated with human gamma globulin (LFT). Thirty-two patients had rheumatoid arthritis which was evaluated functionally according to the classification of Steinbrocker and Treager. Forty-five patients had collagen and other diseases with associated joint involvement, including 3 patients with concomitant rheumatoid disease. Laboratory evidence of inflammatory activity was evaluated according to the presence or absence of C-reactive protein and according to increases in protein-bound polysaccharides associated with certain electrophoretic serum fractions. Sera from rheumatoid arthritis patients (26, or 81%) showed significantly elevated titers (SSC or LFT) as contrasted with a nonrheumatoid group in which only 3 patients with the diagnosis of scleroderma (7%) possessed sera containing high titers. Sera from 5 rheumatic fever patients possessed no agglutinating activity. The order of sensitivity of three different agglutinating tests was as follows: SSC + patients euglobulin < SSC + whole serum < LFT. Occasional sera showed high LF titers in the absence of significant SSC titers. In support of the concept that materials causing agglutination bear no relationship to nonspecific indicators of inflammation, it was found that some rheumatoid sera with marked LF titers contained no C-reactive protein or increased polysaccharide content. However, according to functional classification, a correlation existed between elevated titers and functional disability, although functional activity in turn was not necessarily related to clinical evidences of inflammation.

Latex Fixation Test. IV. Studies on Adsorption of Complement in Rheumatoid Arthritis Sera

By *Jacques M. Singer and Charles M. Plotz.* Arthritis Clinic of the Mount Sinai Hospital, New York.

A study has been carried out to determine the role of complement or a fraction thereof in the agglutination test for rheumatoid arthritis. Both the latex fixation test and a sensitized sheep-cell system were used, and the various components of complement were selectively and specifically inactivated.

The C′4 portion was inactivated with ammonia, methylamine, hydrazine sulfate and chlorhydrate, phenylhydrazine and ether. No effect on either hemagglutination or latex fixation was observed.

C′3 was inactivated by treating sera with zymosan, cobra venom and by rendering the serum deficient in properidin. No effect on either test was observed. After treatment with zymine, however, some inhibition was noted.

When complement was split into midpiece and end piece, agglutination was observed with both, but was greater in the midpiece fraction containing the euglobulin. Inactivation of serum by heating to 56° C. or 63° C., by treatment with the disodium salt

of ethylenediamine tetra-acetic acid (EDTA), by streptokinase-activated plasma, ice storage for a month and prolonged shaking does not destroy serum activity. When sera are treated with sodium citrate, sodium oxalate, sodium azide, sodium fluoride, mercuric chloride, potassium iodide, sodium iodide or potassium cyanate, there is no effect on the latex fixation reaction.

C'1 was adsorbed with bentonite, titanium dioxide, magnesium hydroxide, aluminum hydroxide gel, charcoal, starch and Berkfeld filtration. Amboceptor and the commercial gamma globulin used in latex fixation were similarly treated. Complete adsorption of the agglutinating factor both from serum and amboceptor is noted with bentonite, and partial to complete adsorption is observed following repeated treatments with titanium oxide, kaolin and aluminum hydroxide gel. No effect was noted with Berkfeld filtration, starch or charcoal.

The fact that the agglutinating factor both in rheumatoid arthritis serum and in amboceptor or commercial gamma globulin may be removed is the subject of further investigation.

The Use of Formalized Human Plasma Fraction II in the Study of the Rheumatoid Factor

By *Angelo Taranta*. Irvington House, Irvington, N. Y.

During the course of an immunologic study on formalized proteins it was noticed that sera of rabbits immunized with bovine serum fraction II (BFII) precipitated at consistently and significantly higher serum titers with formalized bovine serum fraction II (FBFII) than with the homologous native antigen. Furthermore, quantitative immunochemical data showed that inhibition of precipitation by excess antigen was strikingly decreased when anti-BFII sera were precipitated with FBFII preparations. These findings prompted an investigation of the effect of formalization in another precipitating system: human plasma fraction II (HFII) and rheumatoid arthritis serum (RAS).

One per cent solutions of HFII in 1/15 molar pH 7 phosphate buffer were treated with various concentrations of formalin at room temperature. The formalized human fraction II (FHFII) preparations thus obtained precipitated with RAS more quickly, at higher serum dilutions and over a wider range of concentrations than the native HFII. This precipitation reaction could be inhibited by native HFII.

A preliminary survey of rheumatoid and non-rheumatoid sera, tested in capillary precipitin tubes with 1% FHFII at 37° C. for two hours suggests the possibility of using a capillary precipitin reaction with FHFII in the detection of the rheumatoid factor.

Formalization of HFII has also proved useful in the application of technics of precipitin analysis in gels to the study of the rheumatoid factor. Rheumatoid sera failing to react with HFII in an Ouchterlony double-diffusion plate have produced bands of precipitate with FHFII.

A Circulating Anticoagulant as the Primary Manifestation of Lupus Erythematosus

By *Edmund W. Campbell, Louise Desy and William Dameshek*. New England Center Hospital, and the Department of Medicine, Tufts University School of Medicine, Boston. (Aided by a grant from the U.S.P.H.S.)

A 29 year old housewife was admitted with a recurrent hemorrhagic disorder of 11 years' duration, manifested by cyclic episodes of menorrhagia and spontaneous ecchymoses.

Decreased plasma prothrombin activity and a positive serology had been discovered at age 18, followed by recurrent migratory polyarthritis. The patient became asymptomatic during three pregnancies, with simultaneous improvement in the laboratory tests. Radium sterilization was performed at age 25 because of exsanguinating menorrhagia, decreased plasma prothrombin activity and prolonged clotting times; penicillin sensitivity was also noted. At 27, the diagnosis of pericarditis, congenital hypoprothombinemia, acquired AHG deficiency and right ureteral stricture was made.

At admission, in 1956, there were scattered ecchymoses and slight splenomegaly. The serology was positive, gamma globulin elevated and the sedimentation rate very rapid. The clotting times of blood in glass and silicone and of recalcified plasma were prolonged; there was marked impairment of prothrombin consumption and decreased plasma prothrombin activity (one- and two-stage technics). Addition of only 0.2 volumes of patient's plasma to normal plasma prolonged the recalcified plasma time. Five times the amount of prepared prothrombin was required to correct the patient's hypoprothrombinemia as compared with congenital hypoprothrombinemia. The platelet count, thromboplastin generation test, Pro-accelerin, SPCA, fibrinogen, etc., were normal. A mild antithromboplastin was demonstrated.

Steroid therapy completely corrected the coagulation defects, which recurred with omission of therapy. L.E. cells were first discovered soon after institution of therapy.

The development of circulating anticoagulants in lupus erythematosus is becoming more apparent. In this patient, no coagulation factor deficiency was present, but an anticoagulant(s) existed which disappeared during steroid therapy. The marked prolongation of coagulation times is difficult to reconcile with either hypoprothrombinemia or a mild antithromboplastin. The anticoagulant may have acted against formed thromboplastin and, also, as a prothrombin inhibitor.

Prolonged Treatment-Free Remission in Disseminated Lupus Erythematosus following Six-and-a-Half Years of Uninterrupted Corticotropin Therapy

By *William Quitman Wolfson*. Highland Park, Michigan.

This report supplements previous interim reports on a young woman whose illness began after prolonged sunlight exposure in the summer of 1948, and followed a low-grade febrile course with a persistent false positive serology until she was hospitalized for study in May, 1949. In her first hospital month she became rapidly worse. Significant findings included evidence of Libman-Sacks endocarditis, typical urinary sediment of lupus nephritis, L.E. cells in bone marrow, pancytopenia with complete eosinopenia, and extensive hemorrhagic lesions of skin and all mucous membranes accompanied by incoagulable blood and complete absence of erythrocyte sedimentation, findings suggesting afibrinogenemia or very active fibrinolysis. Hyperglobulinemia and a falling true A/G ratio (to 0.5) were present. After her fever had daily exceeded 105°F. for a week and she appeared clinically moribund, corticotropin treatment was begun early in June 1949. This first hospital admission lasted until September 1949 and was extremely stormy; complications included severe hypochloremic alkalosis, hypertension, multiple abcesses, bilateral optic neuritis causing temporary blindness, convulsions and complete alopecia. Management continued difficult through the remainder of the first two years, although she regained 40 pounds of lost weight; appendectomy was necessary in January, 1950, and recurrent Hunner ulcer, hepatitis, and less severe optic neuritis were problems, as were fluctuating dose requirements and maintenance of electrolyte equilibrium. The third year brought the last serious problem, when it was necessary to interrupt pregnancy because of a serious relapse. By then, the patient had been taught the technic of supervised self-regulation of dosage and thereafter the course was slowly and painfully upward. Complete hormonal and metabolic studies conducted before, during, and after therapy suggested development of unusual adrenocortical hypersensitivity to corticotropin at this time. Gradually, daily dosages became smaller and intervals were spaced out to single doses every two or three days. The requirement for therapy ceased in November, 1955 and she has since remained well by both clinical and laboratory criteria. This patient was the first person to complete five years of continuous corticoid therapy for any disorder and is believed to be the first patient to have obtained a stable remission without treatment after such therapy. It cannot indicate how often such results can be obtained, but does indicate their possibility. Possibly important factors in management are growth in size and musculature, pituitary-ovarian relations, thyroid function, and adrenal function.

Interaction between the Serum Factor Responsible for the Lupus Erythematosus Phenomenon and Cell Nuclei and Nucleoprotein

By *Halsted R. Holman*. Rockefeller Institute for Medical Research, New York.

Confirmation has been obtained that the serum factor responsible for the lupus erythematosus phenomenon is a gamma globulin. It has been found to sediment at a rate of approximately 7s.

The factor was removed from serum by absorption at 37°C. with nuclei from calf thymocytes, rabbit leukocytes and human monocytes. It was incompletely eluted from the nuclei on incubation at 56°C. Active eluates of LE factor contained only gamma globulin, as shown by immunologic methods, and all the protein present was precipitated by antiserum to normal gamma globulin. Nuclei which had absorbed the LE factor were readily phagocytized by white cells to form LE cells, whereas normal nuclei unexposed to LE serum were not.

Nucleoprotein extracted from nuclei also absorbed LE factor from serum. When nucleoprotein which had absorbed factor was incubated with white cells, LE cells were formed, but this did not occur with nucleoprotein which had not been exposed to LE serum.

The ability of nuclei to absorb the factor was not impaired by prior exposure of the nuclei to normal serum. However, prior treatment of the nuclei with desoxyribonuclease diminished or abolished their ability to absorb factor, as did treatment with protamine or atabrine. Preliminary evidence indicates that nuclei from which the histone fraction has been removed retain their capacity to bind the LE factor.

These data suggest that there is an interaction between the LE serum factor and nucleoprotein, or possibly desoxyribonucleic acid, and that only nuclei or nucleoproteins which have combined with LE factor can be phagocytized to form LE cells.

THERAPEUTICS

Sequential Analysis in Therapeutic Research

By *James A. Hagans, Carl R. Doering, Mervin L. Clark and Stewart Wolf*. Department of Medicine, University of Oklahoma School of Medicine and V.A. Hospital, Oklahoma City.

The rapid appearance of large numbers of new compounds of potential therapeutic usefulness has emphasized the need for more rapid yet valid technics in the evaluation of the efficacy of pharmacodynamic agents. During World War II, similar need for

quality control in industry was met by the statistical innovation of Wald, which was called sequential analysis. This method has had so far very limited application to experimental studies in medicine.

Sequential analysis differs from traditional technics which require the collection of a sizeable quantity of data from test observations as well as from controls before statistical analysis is attempted. In sequential analysis, testing begins at once after establishment of a base-line of control data suitably tested and shown to represent a satisfactorly normal distribution. The control data, together with arbitrarily selected confidence intervals, are utilized for the construction of a graph. Test observations are then plotted sequentially on the graph until a decision is reached.

The studies reported here cover the application of sequential analysis to 5 widely varying biologic systems in man as affected by 19 different pharmacodynamic agents. The 635 observations on 420 human subjects included ipecac-induced vomiting, urine output following the administration of diuretics, gastric secretion following stimulators and inhibitors, blood glucose levels as affected by various agents and the response of the pupils to mydriatics and miotics. All observations were controlled by placebos administered according to a double-blind systematized randomization technic.

In each series of observations, testing was continued until a sufficient number was obtained to allow for analysis by the more standard statistical technics (t-test, chi square analysis). In each instance the results of sequential analysis were confirmed, indicating its potential value as a tool in therapeutic research.

Acute Doriden Poisoning: The Use of Megimide and Hemodialysis

By *George E. Schreiner, Leonard B. Berman and Renato D. Kovach.* Renal Laboratory, Department of Medicine, Georgetown University Medical Center, Washington, D. C.

Doriden (alpha-ethyl-alpha-phenyl glutarimide) is a widely used hypnotic, sedative and tranquilizer, which is now available to depressed patients. It is excreted in the bile, and overdosage may produce a waxing-waning type of hypotension, areflexia and coma. Sudden death may follow respiratory irregularity. It is chemically similar to beta-methyl-beta-ethyl glutarimide (Megimide), which is an analeptic agent now being used in barbiturate poisoning.

The present study concerns 5 patients with severe, acute Doriden poisoning treated with varying combinations of supportive measures, Megimide and hemodialysis. Estimated ingested doses and blood Doriden concentrations, in grams and mg./100 ml., were: case 1, 7.5 and 2.8; case 2, 10 and 2.6; case 3, 10 and 4.0; case 4, 6 plus and 1.4; case 5, 12 and 3.8. Two patients had apnea on intubation, with subsequent recovery. There was one fatality following sudden apnea occurring 69 hours after ingestion.

It is concluded that Doriden in overdosage can produce severe hypotension and coma. Mild cases can be treated with supportive measures, pressor agents and hydration. Moderately severe cases may show striking response to Megimide titration intravenously. Severely poisoned patients who regress following response to large doses of Megimide should be dialyzed by artificial kidney. Doriden is dialyzable. Since Megimide may increase the blood Doriden level, it should enhance dialysis.

Dialysance of Bromide from Blood and Spinal Fluid

By *George E. Schreiner and Leonard B. Berman.* Renal Laboratory, Department of Medicine, Georgetown University Medical Center, Washington, D. C.

The low atomic weight, extracellular distribution and high plasma concentration of bromide in clinical poisoning have been cited as reasons for considering bromide the "ideal" dialyzable toxin. In the first reported case, Merrill and Weller dialyzed a woman with 23 mEq./L. of bromide in the serum and recovered 166 mEq. in the bath. Her psychosis was unchanged for nine hours but cleared in 24 hours. Except for possible cell binding of bromide, there is no ready explanation for the delay.

Since chloride is concentrated in spinal fluid and bromide is an ionic mimic, we have speculated on the possibility of a choroid plexus barrier retarding the expected rapid diffusion. This hypothesis was tested in a 45 year old housewife who had chronically ingested Bromo-Selzer, Nervine and a tonic containing sodium bromide. She had bizarre symptoms over a period of weeks, leading to an investigation for schizophrenia and brain tumor. Blood bromide concentration was 43 mEq./L., which is in the lethal range. Combined hemodialysis and high pressure filtration were carried out in a disposable twin-coil artificial kidney. Serial blood and bath samples were analyzed, together with timed aliquots from a continuously draining spinal catheter. 475 mEq. of bromide were removed from the patient in four hours. CSF/serum values in mEq./L. of bromide were: Control, 33/43; one hour, 31/28; three hours, 25/14; four hours, 23/9.3; five hours 21/6.3; six hours 14.5/4.5. The patient had some clearing of sensorium, remembering episodes during dialysis, and became completely lucid at 27 hours when blood and spinal fluid had equilibrated. The electroencephalogram reverted to normal. It is concluded that dialysis-filtration is the most rapid method yet devised for the removal of bromide and that delayed diffusion from spinal fluid may explain the delayed clinical response.

Addendum

(Because of the pressure of time in a highly compressed production schedule, the following abstract was inadvertently omitted from the section on KIDNEY, *pp. 203–209).*

The Degree of Granulation of the Renal Juxtaglomerular Apparatus in Relation to Hypertension and Sodium Intake

By *Louis Tobian, Janet Thompson and Robert Twedt.*
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The juxtaglomerular index, which indicates the degree of granulation of cells in the juxtaglomerular apparatus, averages 35 in normal Wistar rats. When hypertension is produced by partially constricting one renal artery, the juxtaglomerular granules double in the ischemic kidney and virtually disappear in the untouched contralateral kidney. If the ischemic kidney is then excised 7 weeks later, the hypertension disappears and juxtaglomerular granules reappear in normal abundance in the previously completely degranulated contralateral kidney. After partial constriction of one renal artery, the juxtaglomerular granules in the opposite kidney disappear; but if a constriction is then placed on the artery of this degranulated contralateral kidney, the juxtaglomerular granules reappear. Another group of rats with one ischemic kidney and a contralateral degranulated kidney was placed on a sodium deficient diet which in half the rats caused the juxtaglomerular granules to reappear in the previously degranulated kidney. This diet also increased granulation in normal rats. Partially constricting the renal artery in a rat with only one kidney will produce hypertension, but the granulation of the lone ischemic kidney remains normal. Adrenalectomy doubles the juxtaglomerular index; desoxycorticosterone and salt cause the granules to disappear while concomitantly producing hypertension. Some rats that had received desoxycorticosterone pellets remained hypertensive long after all pellets had been absorbed. The mean juxtaglomerular index for this group was 7, compared to a value of 25 in a similarly treated group that had become normotensive following the absorption of the desoxycorticosterone pellets ($p = .0001$). Hypertension was produced in other rats by removing one adrenal and enucleating the other. Both the hypertensive and the control groups had one kidney removed and were drinking saline solution. The hypertensive group with adrenal enucleation had a juxtaglomerular index of 7, while the control group with intact adrenals had an index of 20 ($p = .001$).

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TO READERS AND CONTRIBUTORS

An Experiment in Medical Journalism

The policy of Clinical Research Proceedings is not only to give rapid publication to research abstracts, but also to provide a vehicle for kinds of articles which do not ordinarily find places in other publications.

These articles should be informed but brief statements of *views*, and they may cover a wide variety of professional matters. Their subject matter will range from purely scientific questions to discussions of research in general and of the environment in which research is carried on. Some of them will be solicited, but we hope that interested authors will submit such material of their own accord. The views expressed may well be somewhat partisan, and we expect that they will evoke counter-statements by workers who are not in agreement with them. A conscientious effort will be made to publish as many of these statements as space allows in early succeeding issues. Such contributions are of course subject to the customary editorial discretion.

Summary reviews of the usual type and original research communications (apart from abstracts) will not ordinarily be acceptable for the Journal, since numerous publication opportunities for such contributions already exist.

The aim of the policy is to provide a meeting place for medical minds, such that the membership of the American Federation for Clinical Research, and other interested persons, may benefit from the enormous amount of careful thought, unsupported by specific laboratory data, that is now being given to important professional issues by competent and conscientious workers. We would like our content to be often controversial without being contentious, and to point occasionally to worthwhile objectives without crusading.

The Editor and his associates solicit the advice and good will of readers of Clinical Research Proceedings. The development of this experiment in medical journalism must depend on the willingness of competent persons to express themselves in print, and also on the willingness of readers of conviction to write in opposition to or in support of communications in the Journal. Such expression of opinion may take the form of "Letters to the Editor," and it is our hope that there will be an active Correspondence Section. Suggestions for changes in plan or for new activities will be welcomed.—*David T. Graham*